An investigation of recalcitrant organic compounds in leachates

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SCHOOL OF CIVIL ENGINEERING AND THE ENVIRONMENT

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ABSTRACT

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Recalcitrant organic compounds remain a key challenge in landfill leachate management as they are resistant to microbial degradation and have potential to damage the water environment. Conventional leachate characterisation methods are time consuming and limited by their inability to provide compositional analysis. This research therefore investigates the characteristics of recalcitrant organic compounds in leachates and undertakes a feasibility study of the possible use of UV absorption and fluorescence spectroscopy for a rapid and economical compositional analysis of recalcitrant organic compounds. Two laboratory experiments are carried out in this regard.

In one experiment, a laboratory scale aerobic biodegradation is carried out on four untreated and two treated leachate samples collected from two UK MSW landfills (Pitsea and Rainham), and leachates are characterised using conventional methods (COD and DOC) as well as UV absorption and fluorescence spectroscopy. It is found that the leachates which have low organic content are easily biodegradable whereas the leachates which have high organic content are not easily biodegradable. UV and fluorescence spectroscopy allow compositional analysis of recalcitrant organic compounds and show that humic and fulvic compounds are the key components of the recalcitrant organic compounds. A rapid biodegradability assessment of different leachates using these novel techniques is in agreement with conventional method showing that a rapid characterisation of leachates using spectroscopic methods is feasible. It is also found that organic compounds in these leachates are aromatic in nature and the leachates containing large amounts of aromatic compounds, condensed aromatic structures are difficult to degrade. In another experiment, the influence of the solid waste component on the development of recalcitrant organic compounds in leachates is investigated by carrying out an anaerobic biodegradation experiment for fresh waste, composted waste, newspaper waste and synthetic waste. Composted waste contributes significantly to the development of the recalcitrant compounds due to the removal of readily biodegradable organic compounds during composting and hence the increased proportion of biologically resistant compounds during the subsequent anaerobic biodegradation. Newspaper waste also contributes significantly to the presence of recalcitrant organic compounds due to the relatively less resistant cellulose and high lignin present in this waste.

This research validates the application of UV and fluorescence spectroscopy for rapid on-site monitoring of landfill leachates that would help scientist and engineers to assess leachate quality, identify various organic compounds and to optimise leachate treatment processes. The analysis performed on Pitsea and Rainham leachates is a promising step towards developing a database of representative information of characteristics of recalcitrant organic compounds in leachates for different UK landfills. This research also provides an understanding of the composition of leachates from different wastes and it can be conclude that disposals enriched with composted and newspaper waste would favour the development of leachates with high concentrations and condensed aromatic structures of recalcitrant organic compounds in a landfill system.

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List of abbreviations

ACD	Acid Detergent Fibre
ADL	Acid Digestible Lignin
BOD	Biochemical Oxygen Demand
BMP	Biochemical Methane Potential
COD	Chemical Oxygen Demand
CW	Composted Waste
DCOD	Dissolved Chemical Oxygen Demand
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EEM	Excitation-Emission-Matrix
FAS	Ferrous Ammonium Sulphate
FE	Final Effluent
FW	Fresh Waste
F-L	Fulvic-Like
IFE	Inner Filtering Effect
Haz	Hazardous
H-L	Humic-Like
LTP	Leachate Treatment Plant
MSW	Municipal solid waste
MW	Molecular weight
Nap-L	Naphthalene-Like
NDF	Neutral Detergent Fibre
NMR	Non Magnetic Resonance
NW	Newspaper Waste
PAE	Phthalates
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphynyls
PVC	Polyvinyl Chloride
P2	Phase 2

P4	Phase 4
SUVA	Specific UV absorbance
SW	Synthetic Waste
TC	Total Carbon
TCD	Thermal Conductivity Detector
TCOD	Total Chemical Oxygen Demand
TDS	Total Dissolved Solid
TFS	Total Fixed Solid
TIC	Total Inorganic Carbon
TN	Total Nitrogen
TOC	Total Organic Carbon
Trp-L	Tryptophan-Like
TS	Total Solid
TSS	Total Suspended Solid
TVS	Total Volatile Solid
Tyr-L	Tyrosine-Like
UV	Ultra Violet
XOM	Xenobiotic Organic Matter

DECLARATION OF AUTHORSHIP

I, Anika Yunus declares that the thesis entitled ‘An investigation of recalcitrant organic compounds in leachates’ and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where I have consulted the published work of others, this is always clearly attributed;
- I have acknowledged all main sources of help;
- parts of this work have been published as:

1. Yunus, A., Smallman, D. J., Stringfellow, A., Beaven, R., and Powrie, W. (2008) Characterisation of landfill leachate DOM using fluorescence spectroscopy. Proceedings of the Waste 2008 conference, September 16-17, Stratford-upon-Avon, UK

2. Yunus, A., Smallman, D. J., Stringfellow, A., Beaven, R., and Powrie, W. (2008) Characterisation of the Recalcitrant Organic Matter Formed During the Anaerobic Biodegradation of Domestic Waste. Proceedings of the Young Water Professionals conference, April 22-24, University College London, UK

Signed: …………………………….
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I dedicate

To my parents
And to my husband

Chapter 1

Introduction

1.1 Scope of the thesis

Despite recent changes in waste management strategy promoting re-use and recycling, landfill continues to play an important role in solid waste disposal in the UK. At present more than 62% of municipal solid wastes (MSW) in England are disposed of to landfills (defra, 2007). One of the potential problems of landfill is the generation of leachates, primarily due to the inherent moisture of the waste and infiltrating rainwater that passes through the waste layers (Tchbanoglous et al., 1993). Leachate can contain dissolved and suspended organic compounds, ammonia-nitrogen, heavy metals, chlorinated organics and inorganic salts (Wang et al., 2002). Its composition and characteristics depend on the waste composition, climate, hydrogeological factors and the age of the landfills (Kouzeli-Katsiri et al., 1999; Chae et al., 2000; Ozkaya et al., 2006). Its characteristics also vary over time with regard to the biodegradable compounds present (Chu et al., 1994; Ozkaya et al., 2006). Leachate is therefore regarded as a continuum of organic compounds of different molecular weight (MW) and structures including biodegradable low MW organic compounds like carbohydrates, amino acids, organic acids as well as high MW humic and fulvic type molecules (He et al., 2006; Pelaez et al., 2009). As waste degrades, the concentration of low MW biodegradable organic compounds in leachates decreases (Calace and Petronio, 1997) and the proportion of high MW organic compounds increases. These high MW organic compounds are generally resistant to biodegradation in the landfill over time and often known as recalcitrant organic compounds.

An influential component of many recalcitrant organic compounds are known as humic substances (humic and fulvic type molecules) which are heterogeneous mixtures of different organic materials such as aromatic, aliphatic and phenolic components (Artiola-Fortuny and Fullar, 1982; Castagnoli et al., 1990 and Christensen et al., 1998). These substances can significantly affect the accumulation and migration of some priority substances and hence, play an important role in the natural environment. Weis et al. (1989) reported that humic substances can interfere with some other organic compounds and metals affecting the transport of anthropogenic compounds to the groundwater and the bioavailability of pollutants. Kang et al. (2002) suggested that humic substances can significantly affect the behaviour of some pollutants, such as trace metal speciation and toxicity, solubilization and adsorption of hydrophobic pollutants, disinfection by-product formation, aqueous photochemistry, mineral growth and dissolution. On the other hand, humic substances can reduce Hg (II) (mercury) to Hg^o thereby providing a pathway for the mobilisation of Hg in the environment (Stevenson, 1994). Humic-like compounds can also react with the chlorine to produce carcinogenic chloroform and other undesirable halogenated compounds which might create considerable problems as well (Stevenson, 1994).

Leachates are usually treated before being discharged to the receiving water. Various physical/chemical and biological treatment processes have been used for the removal of organic compounds from landfill leachates (Kettunen and Rintala, 1995; Imai et al., 1998; Loukidou and Zouboulis, 2001; Uygur and Kargi, 2004; Kargi and Pamukoglu, 2004; Baig and Liechti, 2001; Monje-Ramirez and Orta de Velásquez, 2004; Bila et al., 2005; Ntampou et al., 2005). However, neither physical/chemical nor integrated physical-chemical-biological treatment processes can completely remove the organic compounds contained in leachates (Germili et al., 1991; Ince et al., 1998; Ozkaya et al., 2006; Bilgili et al., 2008). Although the remaining amount of organic compound after treatment does not necessarily represent non-biodegradable fraction of leachate, it has been generally referred as the ‘hard COD’ or recalcitrant material in the literatures (Ince et al., 1998; Chae et al., 2000; Ozkaya et al., 2006; Bilgili et al., 2008). In the UK, biological processes are generally used for economical leachate treatment (Robinson and Knox, 2003) and hence, considerable amount of recalcitrant organic compounds remains in leachates. Chae et al. (2000), Ozkaya et al. (2006) and Bilgili et al. (2008) reported that in a biological leachate treatment system, the biodegradable fraction of leachate can be removed effectively but the recalcitrant fraction passes through the treatment system almost in an unchanged form. Lema et al. (1988) also reported that the presence of humic substances make the leachate less amenable to biological treatment. Therefore, it is essential to understand the evolution and the chemical and structural composition of recalcitrant organic compounds and/or humic-like components over time. Several studies have characterised leachates from different landfills of varying ages to understand the chemical composition of recalcitrant organics (Kang et al., 2002; Nanny and Ratasuk, 2002; Fan et al., 2006). Similar studies have also been reported for different UK landfill sites (Knox, 1983; Robinson and Grantham, 1988; Williams et al., 2002). However these studies do not provide a chronological and systematic catalogue of the changes in chemical characteristics of these compounds in course of biodegradation. In particular, in UK a systematic comparison of the biodegradation associated evolution of the recalcitrant organic compounds in different landfill sites has not been done. An assessment of the influence of individual waste types on the recalcitrant organic compounds in leachates is also rare in the literature. As far as groundwater and surface water pollution is concerned, a chronological monitoring and characterisation of recalcitrant organic compounds over time is important.

Organic compounds in leachates are usually characterised by several established methods; biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total organic carbon (TOC). These methods estimate the organic compound of leachates by oxidising the constituent organic matter in different ways. In BOD method, biochemical oxidation is used to estimate the amount of organic compounds, whereas in COD method strong oxidising agents are used under acidic conditions (APHA, 1998). TOC method uses enhanced oxidizing ambient such as heat, ultra-violet light and/or strong chemical oxidants. Although these methods simply aim to estimate organic compounds in leachates, due to the diverse characteristics of leachate and the different oxidizing conditions in these techniques BOD, COD and TOC methods do not necessarily give the same estimation. The efficiency of any leachate treatment is generally evaluated by the percentage reduction of BOD, COD and TOC over time. However, these techniques are time consuming and laborious and also require considerable sample preparation (Ellis, 1989). For an investigation on the suitability of leachates for discharge, compositional analysis has also been carried out in the past through the prior separation of sub-compounds using degradation methods and later measurements by spectroscopic methods such as Fourier Transformed Infra-Red (FTIR) and Nuclear Magnetic Resonance (NMR) (Hayes et al., 1989; Kang et al., 2002; Nanny and Ratasuk, 2002; Fan et al., 2006). The degradation methods have disadvantages of lengthy sample preparation, transformations and organic carbon lost. As a result, these techniques of compositional analysis also cannot be accepted as a rapid technique for onsite characterisation of recalcitrant materials in leachates. Therefore, there is a need for simple and fast techniques of leachate characterisation to acknowledge the role played by recalcitrant organic compounds of leachates.

In this respect, UV absorption and fluorescence spectroscopy could provide a rapid method for monitoring and characterizing recalcitrant organic compounds and also for fingerprinting the other organic pollutants in leachates to optimise leachate treatment processes. The major advantages of these techniques lie in their rapidity and versatility, their relatively low running costs and the absence of chemicals and detailed sample preparation. The fluorescence technique is potentially applicable for monitoring a wide range of constituents such as humic substance, polyaromatic compounds, xenobiotic compounds etc. (Senesi et al., 1991). The UV absorption and fluorescence techniques have been widely used in various ecosystems (e.g. marine, Coble, 1996; groundwater, Baker and Lamont-Black, 2001; sewage-impacted rivers, Baker, 2001; wastewater and effluents, Westerhoff et al., 2001 and Her et al., 2003). These studies suggest that UV absorption and fluorescence techniques have a significant potential in landfill leachate research by fingerprinting various organic compounds as well as analysing compositional characteristic of the recalcitrant organic compounds in leachates. However, very little work on the characterisation of leachates using these techniques has been carried out (Baker and Curry, 2004; Huo et al., 2008; Ham et al., 2008).

In this work, an analysis of the characteristics of recalcitrant organic compounds in leachates and a feasibility study on the application of UV absorption and fluorescence spectroscopy for compositional analysis of leachates are performed. The aims of the research are to investigate the following.

1. The nature of the recalcitrant organic compounds contained in leachates, particularly its removal in the course of an aerobic biological treatment of leachates collected from two UK MSW landfills (Pitsea and Rainham) and its evolution in the leachates during anaerobic biodegradation of solid waste components to understand the influence of different types of waste on the recalcitrant organic compounds.

2. The use of UV absorption and fluorescence spectroscopy as a rapid and economical technique for compositional analysis of the recalcitrant organic compounds with the aim of assisting and enhancing routinely used analytical methods.

As such, this research performs a systematic comparison of the biodegradation associated evolution of the recalcitrant organic compounds of leachates from two famous UK landfills (Pitsea and Rainham) and from leachates generated during biodegradation of different waste components. These investigations are carried out for the first time using a combination of conventional methods and potentially new techniques of UV absorption and fluorescence spectroscopy. This enriches fundamental knowledge of the influence of solid waste components on the recalcitrant organic compounds and provides deep insight into the characteristics of these compounds on the investigated UK landfill sites/phases that could be used to foresee the necessary treatment in different landfill sites. The combined analysis using conventional methods and UV/fluorescence spectroscopy performs a feasibility study on the use of UV absorption and fluorescence spectroscopy for a rapid and onsite characterisation of recalcitrant organic compounds which might find application if regulatory constraints could be fulfilled. .

1.2 Organization of the thesis

This thesis is divided into six chapters. In this Introduction (*Chapter 1*) the main objectives of this research are outlined (section 1.1).

Chapter 2 makes a detailed review of the literature relating to the origin and removal of recalcitrant organic compounds by various leachate treatment processes. This chapter reviews the applications and limitations of conventional methods for characterisation of recalcitrant organic compounds of leachates. Potentially useful techniques of UV absorption and fluorescence spectroscopy are also presented in this chapter with a focus on effective and rapid monitoring and characterisation.

Chapter 3 provides the analytical methods and experimental procedures used to investigate the nature and composition of the recalcitrant organic compounds in landfill leachates and also in leachates generated during anaerobic biodegradation of components of solid waste.

Chapter 4 summarizes the experimental results of the characteristics of the recalcitrant organic compounds in leachates in the course of an aerobic biological treatment process. Changes in the composition of the recalcitrant organic compounds of leachates during laboratory scale aerobic biological treatment system was characterised by the conventional COD and DOC measurements and also by UV absorption and fluorescence spectroscopy.

Chapter 5 summarizes and discusses the experimental results of the characteristics of leachates formed during an anaerobic biodegradation of waste components. Conventional COD and DOC measurements and UV absorption and fluorescence spectroscopic results are reported to evaluate the development of recalcitrant organic compounds in the associated leachates as the waste degrades.

Chapter 6 proposes the major conclusion drawn from this research, followed by recommendations for future work.

Chapter 2

Background

2.1 Introduction

This chapter presents background studies on the recalcitrant organic compounds in landfill leachates. The origin, composition and the removal of recalcitrant organics by various leachate treatment processes are reviewed. The applications and limitations of available methods to estimate the total amount of recalcitrant organic compounds and to fingerprint the constituent compounds of recalcitrant organics are discussed. The possible application of UV absorption and fluorescence spectroscopy in characterising the recalcitrant organics is assessed by studying published reports of UV and fluorescence applications to marine and estuarine waters, freshwater, and wastewaters.

2.2 Leachate generation in landfill

Leachates are generated from municipal solid wastes (MSW), which are a heterogeneous mixture of residential, commercial and some industrial wastes. When disposed of to landfills, the organic components of the MSW are degraded by a combination of physical, chemical and microbial processes (Kouzeli-Katsiri et al., 1999; Tchbanoglous et al., 1993). This leads to the generation of leachates, primarily due to the inherent moisture of the waste, the degradation process and infiltrating rainwater that pass through the waste layers (Tchbanoglous et al., 1993). Leachate is a highly contaminated and heterogeneous wastewater and contains dissolved and suspended organic compounds, ammoniacal-nitrogen, heavy metals, chlorinated organics and inorganic salts (Wang et al., 2002). The composition and characteristics of landfill leachates depend on the nature of the disposed waste, hydrogeological factors, climate and the age of the landfills (Kouzeli-Katsiri et al., 1999; Chae et al., 2000; Ozkaya et al., 2006). With time a landfill passes through a short aerobic phase followed by a long anaerobic decomposition phase. Several studies have described waste biodegradation in landfill as a five-phase process. These are the aerobic phase, acidogenic phase, acetogenic phase, methanogenic phase and final aerobic phase (figure 2.1) (Lu et al., 1985; Pohland and Harper, 1985; Tchbanoglous et al., 1993; McBean et al., 1995). The breakdown processes in the various phases are potentially complex at the microscopic level, involving hundreds of intermediate biochemical reactions and compounds. Many of these reactions require additional specific synergistic chemicals, catalysts or enzymes. The biodegradation processes and products relating to organic matter at the various phases are described below. The nature of these phases plays a significant role in the composition of leachates.

Lu et al. (1985) and McBean et al. (1995) reported that in the *aerobic degradation* phase, aerobic micro-organisms metabolise oxygen in the air trapped within the waste and a proportion of the organic fraction of the waste to produce carbon dioxide (CO_2), water (H_2O) and partly degraded residual organics. Leachate produced during this phase is likely a result of the moisture squeezed out of the waste during compaction and cell construction (Lu et al., 1985). It is characterized by the constituent suspended particulate matter, the dissolution of highly soluble salts initially present in the landfill, and the presence of small amounts of organic species from aerobic biodegradation (Lu et al., 1985; McBean et al., 1995). The released energy is in the form of heat and can raise the temperature to 70-90^{o}C (Lu et al., 1985; McBean et al., 1995). This stage can last for several days or weeks depending on the availability of oxygen.

In the second stage of the biodegradation process, anaerobic and facultative micro-organisms hydrolyse and ferment cellulose and/or other materials (proteins, fats etc.) making them more readily available for the acidogenic bacteria. Acidogenesis is characterised by the production of acetic acid from the monomers produced in the preceding stage and other volatile fatty acids which are derived from the biodegradation of protein, fat and carbohydrate components of the waste (Lu et al., 1985; McBean et al., 1995). The temperature within the landfill falls to 30-50^{o}C (WMP 26B, 1995). Carbon dioxide and hydrogen may rise up to 80% and 20% of total volume of gas respectively (WMP 26B, 1995). The production of different by-products depends on composition of waste, the environmental condition and the presence of different bacterial species.

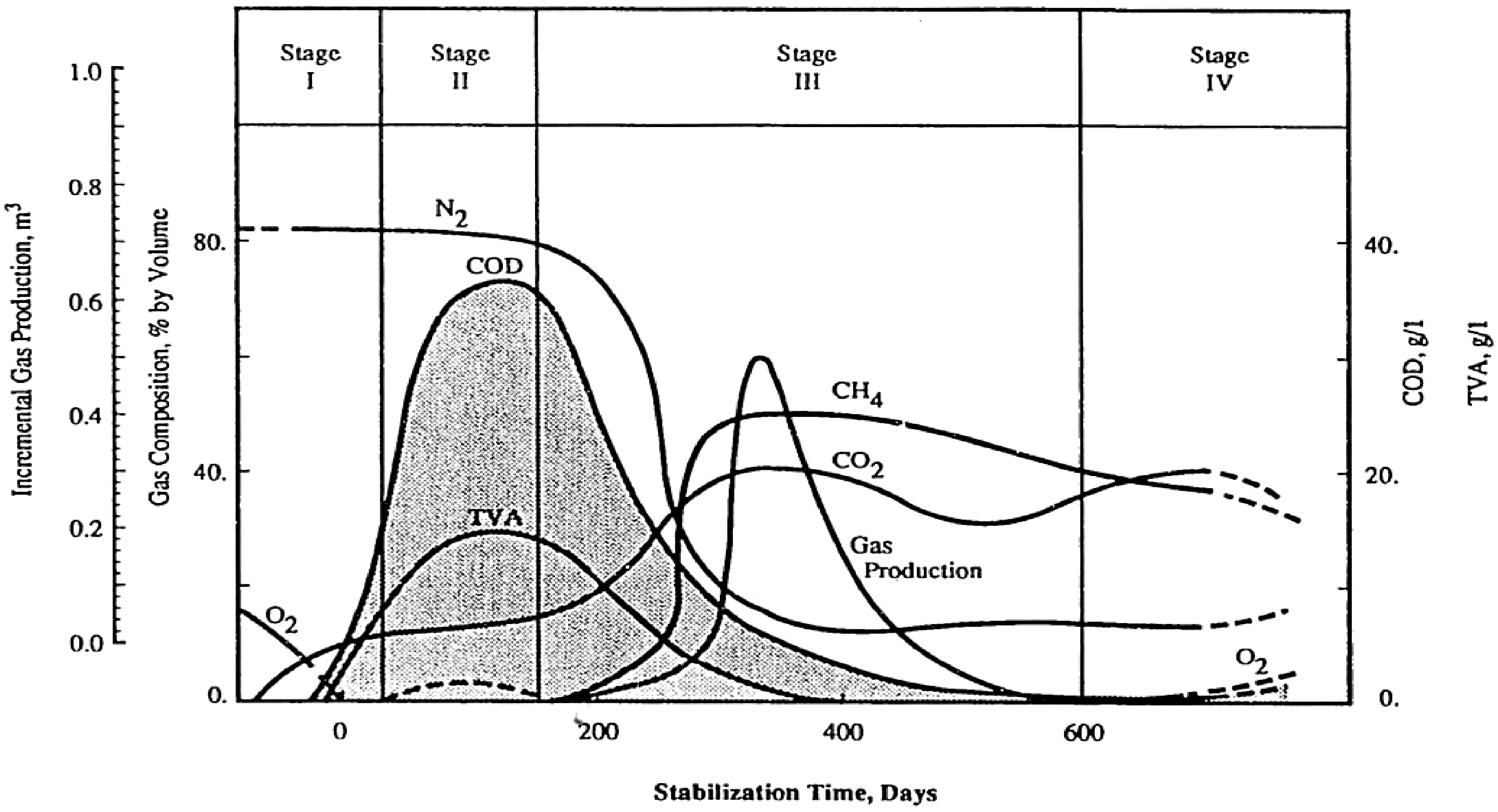


Figure 2.1 Changes in selected parameters during the phases of landfill stabilization (Pohland and Harper, 1985)

- **Phase V is not shown**
- **COD – Chemical Oxygen Demand**
- **TVA – Total Volatile Acids**

In the a*cetogenesis* phase, *acetogenic bacteria* convert the long chain fatty acids (propionic, butyric, valeric and caproic acids) to acetic acid, carbon dioxide and hydrogen. Lo (1996) reported that the leachate produced in the acidogenic and acetogenic phases is characterised by an acidic pH (typically 5.0 or 6.0), high concentration of biodegradable organic compounds and an unpleasant smell. Also because of the low pH values in the leachate, a number of inorganic compounds, principally heavy metals could be solubilised during this phase resulting in a potentially chemically toxic leachate. Hydrogen sulphide may also be produced in this stage as the sulphate compounds in the waste are reduced to hydrogen sulphide by sulphate reducing micro-organisms (Christensen et al., 1996).

In the following *methanogenic* phase, the simple organic compounds released by the acetogenic processes start to be consumed by the methanogenic bacteria to produce methane and carbon dioxide. The leachate produced in this phase is characterised by a range of pH of 6.8 to 8.0 (Zehnder, 1978), low concentrations of biodegradable organic compounds and high levels of ammonia nitrogen (NH_3-N) (Lo, 1996). Acetogenic and methanogenic bacteria form a symbiotic (mutually beneficial) relationship. Acetogenic bacteria depend on the methanogenic bacteria to remove hydrogen, the accumulation of which leads to the suppression of acetogenesis whereas methanogenic bacteria depend on acetogenic bacteria to provide the acetic acid and hydrogen required for methane production. As methanogenic bacteria are slow growing bacteria, they are unlikely to be present in the early stage of waste biodegradation. Their initial absence would lead to the increased partial pressure of hydrogen, which would in turn result in the inhibition of acetogenesis and accumulation of long chain fatty acids thus lowering the pH, leading to the further inhibition of methanogenesis.

An additional aerobic phase of biodegradation has been proposed by Christensen and Kjeldsen (1995). They stated that the final aerobic stage of waste degradation is initiated by the aerobic micro-organisms which replace the anaerobic forms, and becomes re-established when the rate of oxygen diffusion through the capping from the atmosphere becomes greater than the methane and carbon dioxide produced by anaerobic degradation.

2.3 Landfill leachate characterisation and the origin of recalcitrant organic compounds

Landfill leachates are usually characterised by several methods including biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC). These methods simply aim at estimating organic compounds in leachates by oxidising the constituent organic matter and hence, the efficiency of any leachate treatment is evaluated by the percentage reduction of the BOD, COD and TOC over time. The amount of organic compounds which are eventually not removed in the course of a treatment processes is considered as recalcitrant organic compounds and usually estimated by the remaining percentage of BOD, COD and TOC. The detail processes of BOD, COD and TOC techniques are discussed below:

2.3.1 Biochemical Oxygen Demand (BOD)

The BOD is essentially a measure of the amount of oxygen required for the biochemical oxidation of organic compounds usually over 5 to 20 days (Brookman, 1997). In the BOD test, micro-organisms are used to oxidise and convert the carbonaceous organic matter to carbon dioxide and water. The presence of toxic substances in leachates can effect (or even kill) the micro-organisms, even at low concentrations. In addition, the heavy metals such as lead, copper, mercury or cadmium can inhibit or sometimes completely prevent the oxidation of the organics present in the leachates (Bourgeois et al., 2001). However, the BOD test is slow to yield information and takes a relatively long time to complete.

2.3.2 Chemical Oxygen Demand (COD)

The COD is a measure of the oxygen equivalent of the organic matter that is susceptible to oxidation by a strong chemical oxidant (Bourgeois et al., 2001). The COD test uses potassium dichromate ($K_2Cr_2O_7$), acid and heat to oxidise organic carbon to carbon dioxide and water. The amount of oxidizable organic matter, measured as oxygen equivalent, is proportional to the potassium dichromate consumed. The major advantage of the COD test is that the results can be obtained within a relatively short time (approximately 2 hours). Additionally the presence of toxic substances does not effect COD measurement. One of the main limitations of the COD test is the inability to differentiate between the biodegradable and non-biodegradable organic compounds. In addition, the presence of chloride, bromide etc. in the leachates interfere in the COD test, however added silver nitrates reacts with chlorides, bromides or iodides to produce precipitates.

2.3.3 Total Organic Carbon (TOC)

The TOC test uses heat, ultra-violet light or a strong chemical oxidant (or a combination of all three) to oxidise the organic matter. The advantages of TOC method over BOD and COD are as follows:

1. Quick and easy
2. Chloride and bromide do not interfere
3. Not harmful
4. No requirement of hazardous chemicals (chromium, silver nitrate)
5. No micro-organisms involved

A relative comparison of these three methods is presented in Table 2.1

Table 2.1 A relative comparison among three methods (BOD, COD, TOC)

	(BOD)	(COD)	(TOC)
Analysis technique	Determination of O_2 consumption due to biochemical oxidation of sample	Determination of O_2 consumed during chromic acid digestion of sample	High Temperature Combustion (HTC) detection of CO_2 analysis with the removal of inorganic carbon
Reagents required	Bacterial seed	Chromic acid, silver sulphate, sulfuric acid, ferrous ammonium sulphate	Phosphoric acid
Time per analysis	5 to 20 days	2-3 hours	4 – 6 minutes
Accuracy	5-8%	5-8%	2-3%
Interferences	Inorganic carbon	Nitrite, Sulfide, Chlorides, Fe, Acids, others	Toxic substances

The chemical composition of leachates characterised by BOD, COD and TOC has been studied extensively by various researchers in the past few decades (Qasim and Burchinal, 1970; Chian and DeWalle, 1976; Pohland and Englebrecht, 1976; Lu et al., 1985; Lema et al., 1988; Chu et al., 1994; Ragle et al., 1995; Chen et al., 1996; Lo, 1996; Christensen et al., 2001). It is therefore well known that there is a decreasing trend of BOD, COD, TOC and the ratio of BOD/COD with increasing age of the landfill. Consequently old landfill leachates have low biodegradability and are correlated with low BOD and COD values and low BOD/COD ratios. Calace et al. (2001) also reported that the young landfill leachates have a high fraction (70%) of low molecular weight (< 500 Dalton) and a low fraction (18%) of high molecular weight (>10000 Dalton) compounds. Conversely old landfill leachates have low and high molecular weight fractions of about 28% and 67% respectively. This means that as landfill degradation progresses, the concentration of low molecular weight biodegradable organic compounds in leachates decreases due to the fermentation of hydrolysable organic compounds (Calace and Petronio, 1997) and high molecular weight organic compounds are increased. These high molecular weight organic compounds are microbially refractory under the preceding condition in the landfill over time (Chian and DeWalle, 1976; Göbbels and Püttmann, 1996; Calace and Petronio, 1997) and are often known as recalcitrant organic compounds (Germili et al., 1991; Ince et al., 1998; Ozkaya et al., 2006).

The generation of recalcitrant organic compounds depends significantly on the components of the solid waste disposed of to the landfills such as cellulose, hemi-cellulose, lignin, protein and lipids. Stevenson (1994) suggested that lignin is the main source of recalcitrant compounds. Tong et al. (1990) reported that lignin comprises a complex polymer of phenylpropane units, which are cross-linked to each other with a variety of different chemical bonds and are especially resistant to anaerobic biodegradation. However, cellulose and hemi-cellulose, which are usually biodegradable in aerobic and anaerobic environments (Tong et al., 1990), are also accepted to be recalcitrant compound precursors (Varadachari and Ghosh, 1984; Inber et al., 1989). This was explained by Komilis and Ham (2003) who reported that the retardation of aerobic and anaerobic biodegradation of cellulose and hemi-cellulose is primarily due to the sheathing of cellulose and hemi-cellulose by lignin. This agrees with Khan (1977) who performed a laboratory experiment on the degradability of cellulose under anaerobic conditions. He showed that about 40%, 90% and 97% of cellulose in newspaper, office paper and Whatman filter paper were degraded. This was due to the fact that newspaper was highly lignified whereas office paper and Whatman filter paper were nearly delignified. Tuomela et al. (2000) reported that lignin degradation is primarily an aerobic process and white-rot fungi are responsible for most of the lignin decomposition. In an anaerobic environment such as in landfills, lignin can persist for years and its presence can significantly reduce the bioavailability of cellulose and hemi-cellulose (Rees, 1980; Wang et al., 1994; Komilis and Ham, 2003).

Microbial re-synthesis is also an important source of non-biodegradable or recalcitrant compounds. Pichler and Kogel-Knabner (2000) found that both protein and lipids are re-synthesized microbially in the MSW environment, resulting in long chain high molecular weight compounds which are poorly degradable. Dinel et al. (1996) also reported that proteins may be preserved by encapsulation into recalcitrant cell wall polymers of micro-organisms and become resistant to biodegradation.

2.4 Composition of recalcitrant organic compounds and its effect on the environment

Recalcitrant materials in landfill leachates consist mostly of humic substances (Artiola-Fortuny and Fullar, 1982; Castagnoli et al., 1990 and Christensen et al., 1998). On the basis of the solubility, Schnitzer and Khan (1972) subdivided humic substances into humic acids (HA, soluble in basic medium but not soluble in acid medium), fulvic acids (FA, soluble both in acid and basic medium) and humin (insoluble in water under any pH condition). Hayes et al. (1989) and Stevenson (1982, 1994) reported that aromatic structures are the dominant building blocks of the structural core of humic substances and the compositions of humic substances are dominated by structural moieties (benzene rings, aliphatic segments, hexose, pentose, and amino acids), functional groups (carboxyl, carbonyl, hydroxyl, amine, phenolic) and linkages (ester, amide and ether). While these studies provided some information on the presence of several structural moieties, functional groups, linkages as well as identifying other aromatic and aliphatic components among the degradation products, there was no report in the literature revealing any unique molecular structure that could be considered as an essential building block of humic substances. It appears that molecules in humic substances differ from one another in chemical and physical properties. Consequently, humic substances are complex mixtures of macromolecules of varying molecular size, structure and chemical functionality. Because of this versatility, these substances can significantly affect the accumulation and migration of some priority substances and hence, play an important role in the natural environment (Weis et al., 1989; Kang et al., 2002)

Stevenson (1994) stated that these compounds are not physiologically harmful but are aesthetically unacceptable because they impart a reddish-black colour to potable water and lakes. Their presence can have both negative and positive impacts. Weis et al., (1989) reported that humic substances can interfere with some other organic compounds and metals affecting the transport of anthropogenic compounds to the groundwater and the bioavailability of pollutants. Kang et al. (2002) stated that humic substances significantly affect the behaviour of some pollutants, such as trace metal speciation and toxicity, solubilization and adsorption of hydrophobic pollutants, disinfection by-product formation, aqueous photochemistry, mineral growth and dissolution. These compounds can also affect photochemical processes; reduce Hg (II) (mercury) to Hg^o thereby providing a pathway for the mobilisation of Hg in the environment (Stevenson, 1994). Humic-like compounds can also react with the chlorine to produce carcinogenic chloroform and other undesirable halogenated compounds which might create considerable problems as well (Stevenson, 1994). It is therefore essential to understand the evolution and the chemical and structural composition of recalcitrant organic compounds and/or humic-like components over time.

2.5 Effect of treatment on removal of recalcitrant organic compounds

Leachates are usually treated before being discharged to the receiving environments. Various physical, chemical and biological treatment processes have been used extensively for the removal of organic compounds from landfill leachates. These methods have been reviewed in detail by Renou et al., (2008).

Table 2.2 summarizes some of the aerobic and anaerobic biological processes and their efficiency in removing organic compound from landfill leachates. The efficiency of a treatment process is evaluated by the percentage removal of the BOD, COD and TOC. It can be seen from Table 2.2 that with the exception of Kettunen and Rintala (1995), a treatment efficiency of about 80-90% has been achieved by aerobic biological treatment processes. The reported anaerobic treatment efficiency is about 65-75%. Kargi and Pamukoglu (2004) also reported that it is advantageous to carry out anaerobic treatment prior to an aerobic process for leachates containing a high COD. Using this approach Kettunen et al. (1996) achieved a COD removal of 80-90%. It is worth mentioning that the treatment efficiencies presented in Table 2.2 cannot be directly compared with each other due to the initial differences in COD values. However, it is generally found that about 10-20% recalcitrant organic compounds measured by BOD, COD and TOC remain after biological treatment.

Table 2.2 Studies on landfill leachate biological treatment (aerobic and anaerobic)

Reference	Initial leachate COD (mg/l)	Treatment	Overall results (COD, BOD, TOC removal)
Gourdon et al. (1989)	850-1350	Aerobic (Trickling filter)	87% (BOD)
Maehlum (1995)	1182	Aerobic (lagooning)	89% (COD)
Kettunen and Rintala (1995)	2000-3000	Aerobic (Moving Bed Biofilm Reactor)	46-64% (COD)
Loukidou and Zouboulis (2001)	5000	Aerobic (Moving Bed Biofilm Reactor)	81% (COD)
Zoubouli et al. (2001)	15000	Anaerobic (Sequencing batch reactor)	75% (COD)
Kettunen et al. (1996)	1800	Sequential anaerobic-aerobic biological reactor (SBR)	80-90% (COD)
Kettunen and Rintala (1998)	1500-3200	Anaerobic (Up-flow anaerobic sludge blanket reactor)	65-75% (COD)

Imai et al. (1998) reported that a single, conventional biological treatment is not effective in treating leachates with a high concentration of organic matter resistant to biodegradation and this type of leachate needs additional treatment to become more biodegradable. According to Imai et al. (1998), one way of doing this is to employ physical-chemical processes as a pre-treatment prior to a biological treatment or as a final polishing treatment. Table 2.3 summarizes the efficiencies of some of these treatments. It can be seen that using granular or powder activated carbon (PAC) as an adsorbent a treatment efficiency of around 90% has been achieved (Morawe et al., 1995; Welander and Henrysson, 1998; Rivas et al., 2003; Kargi and Pamukoglu, 2004). This indicates that adsorption was effective as a pre-treatment method for the COD removal.

Table 2.3 Studies on landfill leachate integrated physical-chemical-biological treatment

Reference	Initial leachate COD (mg/l)	Treatment	Overall results (COD, BOD, TOC removal)
Morawe et al. (1995)	879-940	Granular activated carbon as adsorbent	91% (COD)
Welander and Henrysson (1998)	800-2000	Powdered activated carbon as adsorbent	96% (TOC)
Kargi and Pamukoglu (2004)	7000	Powdered activated carbon as adsorbent	90% (COD)
Rivas et al. (2003)	7400-8800	Activated carbon as adsorbent	90% (COD)
Bila et al. (2005)	3096	$FeCl_3$ or $Al_2(SO4)_3$ as coagulant	73% (COD)
Ntampou et al. (2005)	1000	$FeCl_3$ or Al_2Cl_3 as coagulant	89% (COD)
Monje-Ramirez and Orta de Velásquez (2004)	5000	$Fe_2(SO4)_3$ or Al_2O_3 as coagulant	78% (COD)

Amokrane et al. (1997) also suggested coagulation as a pre-treatment prior to a biological process. Aluminum sulfate, ferrous sulfate, ferric chloride and ferric chloro-sulfate were commonly used as coagulants (Amokrane et al., 1997; Bila et al., 2005). The removal of organics by coagulation is associated with the removal of humic substances (Ntampou et al., 2005). There are two mechanisms of removal of humic substances by coagulation; (1) binding of cationic metal species to the anionic sites resulting in the neutralisation of humic substances and reduction of their solubility and (2) adsorption of humic substances onto the produces amorphous metal hydroxide precipitates (Ntampou et al., 2005). A treatment efficiency of 70-80% has been achieved by coagulation (Monje-Ramirez and Orta de Velásquez, 2004; Bila et al., 2005; Ntampou et al., 2005).

Several researchers have investigated the efficiency of ozonation (O_3) for the treatment of landfill leachates (Huang et al., 1993; Monje-Ramirez and Orta de Velásquez, 2004). Monje-Ramirez and Orta de Velásquez (2004) reported that chemical oxidation with O_3 makes possible the transformation of recalcitrant material into biodegradable forms or CO_2. Table 2.4 shows the achieved efficiencies in various leachate treatments with O_3. In sanitary landfill leachates, oxidation with O_3 is not very effective as a single process and the efficiency is only 30-40%. However, in combination with other methods (biological, coagulation), O_3 enhances the overall efficiency of COD removal, from 44% to 97%.

The above studies suggest that physical-chemical methods as a pre-treatment or final polishing are sometimes more effective than single biological methods in removing recalcitrant organic compounds from leachates. Physical-chemical methods are suitable for small scale laboratory based experiments but are costly in terms of equipment and infrastructure investments, energy requirement, and large scale chemical usage for landfill applications and hence they are not economically viable. As a result, biological processes are the most commonly used methods of leachate treatment in the UK (Robinson and Knox, 2003), hence considerable amount of recalcitrant organic compounds can remain in landfill leachates. Therefore, there is a need to understand the characteristics of recalcitrant organic compounds in leachates. In particular it is essential to understand the recalcitrant organic compounds in leachates over time and with the nature of waste components. There appears to have been little if any formal study of this, although information on the composition of leachates collected from landfills of different ages is given by Kang et al. (2002); Nanny and Ratasuk (2002) and Fan et al. (2006). It was mentioned before that leachate composition varies significantly with climate, hydrogeological factors and the nature of the waste disposed in a landfill. Thus these studies of leachates from different landfills of varying ages do not form a chronological and systematic catalogue of the composition of recalcitrant organic compounds with time. As far as groundwater and surface water pollution is concerned, the systematic characterisation and monitoring of landfill leachates is imperative.

Table 2.4 Studies on landfill leachate treatment with O_3

Reference	Leachate type and COD (mg/l)	Treatment	Overall results (COD removal)
Huang et al. (1993)	Old leachate (1610)	O_3	44%
Imai et al. (1998)	Old leachate (126)	O_3	33%
Baig and Liechti (2001)	Young leachate (1585)	Coagulation ($FeCl_3$) + Aerobic treatment + O_3	94%
Monje-Ramirez and Orte de Velasquez (2004)	Coagulation treated leachate (1058)	Coagulation + O_3	78%
Schulte et al. (1995)	Biologically treated leachate (760)	O_3	97%
Bila et al. (2005)	3096	$FeCl_3$ or $Al_2(SO4)_3$ as coagulant + O_3 + Biological treatment	73%
Ntampou et al. (2005)	1000	$FeCl_3$ or Al_2Cl_3 as coagulant + O_3	89%

2.6 Limitation of conventional characterisation methods (BOD, COD, TOC)

As discussed before, in the leachate treatment processes the amount of organic compounds that can not be removed in the course of a treatment is described as the recalcitrant organic compounds and can be detected by the remaining percentage of BOD, COD and TOC. The disadvantages of these standard characterisation techniques (BOD, COD and TOC) of leachates include lack of reliability and the difficulty in achieving reproducible results. Though COD and TOC test results can be obtained within a relatively short time (2 to 3 hrs), BOD test takes a long time (5 to 20 days). Additionally, all these techniques are laborious and also require sample preparation (Ellis, 1989). Therefore, these conventional techniques do not provide a method for rapid and effective monitoring of recalcitrant organic compounds in leachates over a time frame that could influence the leachate treatment system. Therefore, it would be economical if rapid characterisation techniques of recalcitrant organic compounds could be developed. In addition, these standard techniques can only be used to estimate the amount of remaining recalcitrant organic compounds in leachates in the course of a treatment (Tables 2.2, 2.3 and 2.4) but they cannot be used to identify the degradation nature of some constituents of recalcitrant organic compounds.

2.7 Compositional analysis of recalcitrant organic compounds and/or humic substances

Several techniques which were applied in the past for characterizing different components of humic substances (Schnitzer and Khan, 1978; Stevenson, 1982, 1994; Hayes et al., 1989a; Weis et al., 1989; Calace and Petronio, 1997; Christensen et al., 1998; Kang et al., 2002; Nanny and Ratasuk, 2002; Fan et al., 2006) are summarized in Table 2.5.

Elemental composition of humic substances was usually investigated in the past using chemical/physical or XPS method. Both of these methods have the disadvantage of considerable sample preparations. XPS has been well established as a powerful tool for the characterization of surfaces and surface reactions in heterogeneous catalysis, in corrosion science (Briggs and Seah, 1990), wood science (Johansson et al., 1999) and soil sciences (Yuan et al., 1998). However, for characterizing the aquatic humic substances in leachates by XPS method, a number of experimental problems had to be solved. In particular, reproducible preparations of thin solid layers of dissolved humic substances on an appropriate substrate surface and the homogeneity of such layers are key challenge of XPS for this application.

Size and shape of humic particles is usually characterized by Electron Microscopy although it has been reported that estimation using this technique needs extra care due to a strong pH dependency (Chen and Schnitzer, (1989). Gel chromatography has been used for measuring the molecular weight of humic substances (Schnitzer and Khan, 1978). However, this method has been reported to overestimate the molecular weight due to gel-solute interactions (Schnitzer and Khan, 1978).

Table 2.5 Common methods for the characterization of humic substances

Method		Information	Reference
Chemical/ Physical	Elemental analysis	Elemental composition (C, H, N, S, O)	Christensen et al. (1998); Kang et al. (2002); Nanny and Ratasuk (2002); Fan et al. (2006)
	Microscopy	Size and shape	Chen and Schnitzer (1989)
	Acid/Base titration	Proton capacity	Perdue (1998); Abbt-Braun and Frimmel (2002)
	Thermal degradation	Pyrolysis products	Abbt-Braun et al. (1989); Schulten et al. (2002)
	Oxidative, reductive	Degradation products	Christman et al. (1989)
Spectroscopy	X-ray photoelectron spectroscopy (XPS)	Elemental composition (C, H, N, S, O)	Bubert et al. (2000)
	Fourier Transformed Infra-red (FTIR)	Qualitative determination of functional groups	Hayes et al. (1989)
	Nuclear Magnetic Resonance (NMR)	Quantitative determination of functional groups: aromatics, aliphatics, carbohydrates	Kang et al. (2002); Nanny and Ratasuk (2002); Fan et al. (2006)
Chromatography	Gel chromatography	Molecular size, weight	Perminova et al. (1998); Schmitt-Kopplin et al. (1998)

Acid/Base titration methods have also been employed for the determination of acidic functional groups in the humic substances (Perdue, 1998; Abbt-Braun and Frimmel, 2002). However, the dissociation of two major types of functional groups; carboxyl groups (-COOH) and hydroxyl group (-OH) were reported to be difficult by this method.

In addition to the characterizing elemental composition and size/shape of humic particles several methods are also available in the literature for characterizing constituent organic compounds and/or functional groups in humic substances (Table 2.5). Since the early to mid-1980s, degradation methods have been applied to produce identifiable sub-compounds of humic substance for estimation. Thermal pyrolysis, oxidative-reductive and hydrolytic methods are often used as degradation reactions for sample preparation. Thurman and Malcolm (1981) also used a method for extraction and isolation of humic substances from leachate dissolved organic carbon (DOC) for chemical characterisation which was a variant of degradation method. This method involves the use of XAD resin for the extraction of humic substances from leachate DOC and the acidification of humic substances to pH 1.0 for the isolation of humic and fulvic acids. However, there is always the question of unknown denaturing reactions and of transformations during the degradation procedure. This sometimes leads to different degradation products formed from one single precursor due to the involvement of several treatments with acids and bases to extract humic substances. Smith et al. (1991) reported that during these procedures a substantial part of DOC (about 30%) may also be lost.

In recent years, FTIR and NMR studies have become the most important spectroscopic methods of structural characterization of humic substances for identifying various functional groups such as quantification of partial structures such as aliphatic, O-alkyl, aromatic and carboxyl carbons. FTIR characterisation is mainly qualitative and NMR method although provides quantitative information of some functional groups, the heterogeneous nature of humic substances often results in many overlapping signals that complicate the interpretation. As a result, to get an unambiguous estimation, these methods were usually applied after prior separation of identifiable sub-compounds. For example, Christensen et al. (1998) fractionated hydrophilic fraction of leachate DOC using the method developed by Thurman and Malcolm (1981) and characterised the hydrophilic fraction by size-exclusion chromatography, elemental analysis, acid-base titration and FTIR. It was concluded that although hydrophilic fraction resembles, in many ways to the humic and fulvic acids a substantial portion was also found to be neither humic nor fulvic acid indicating the potential difficulty of degradation methods.

The qualitative and quantitative characterisation of non-humic fractions such as carbohydrates, amino acids in humic substances has also been studied in the past. Frimmel et al. (2002) and Jahnel et al. (2002) fractionated humic substances by hydrolysis with acids, bases and enzymes and analysed by ^{13C}NMR. They showed that monosaccharides, like pentoses, hexoses, deoxycarbohydrates and aminosugars make up to 8 mass percent of the organic carbon of fulvic and humic acids. ^{13C}NMR analyses showed that up to 30% carbon in humic substances is involved in carbohydrate structures whereas ^{15N}NMR data showed that up to 90% of nitrogen is attributed to amide nitrogen (Jahnel et al., 2002).

In addition to above mentioned techniques there are two other techniques of compositional analysis of humic substances which is discussed in the next section.

2.8 Proposed technique of leachate characterisation

The discussions in section 2.6 imply that the conventional methods of leachate characterisation (BOD, COD and TOC) require considerable sample preparation and cannot perform the complete compositional analysis of leachates. Among the different methods of compositional analysis discussed in section 2.7, degradation methods such as thermal pyrolysis, oxidative-reductive, hydrolysis and/or spectroscopic methods such as FTIR and NMR could be used to investigate the evolution of some constituent organic compounds of recalcitrant materials in course of a biodegradation. However, the degradation process itself can be treated as lengthy sample preparation and obviously has the disadvantages such as unknown denaturing reactions, transformations and substantial DOC lost. Although spectroscopic methods FTIR and NMR are interesting, an unambiguous estimation of constituent organics often requires prior separation of identifiable sub-compounds using degradation. As a result, these techniques cannot be accepted as a rapid technique for onsite compositional analysis of recalcitrant materials in leachates. In this respect, UV and fluorescence spectroscopy might be attractive for analyses of leachates which do not require any sample preparation. In this section, some applications of UV and fluorescence spectroscopy in characterising marine and estuarine waters, freshwater, wastewater etc is presented. These studies show that these techniques have potential for additional landfill leachate characterisation. The key advantage is that these techniques have the flexibility of rapid estimation of recalcitrant organic compounds in leachates and have the ability of doing compositional analysis of recalcitrant organics.

2.8.1 UV visible spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis) involves the spectroscopy of photons in the UV-visible region (200-800 nm wavelengths). When sample molecules are exposed to light, some of the light energy is absorbed and the electrons are promoted to a higher energy orbital. An optical spectrometer measures the intensity of light passing through a sample and compares it with the intensity of light before it passes through the sample. The amount of light absorbed by the sample is proportional to the concentration of those organic compounds in the samples which absorb light in the 200 to 800 nm region. Thus the spectrophotometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. An ultraviolet-visible spectrum is essentially a graph of light absorbance versus wavelength in a range of ultra-violet or visible regions.

MacCarthy and Rice (1985) reported that the absorbance of UV light by a molecule is mainly due to aromatic ring structures. The aromatic molecules likely to absorb light in the 200 to 800 nm region are referred to as chromophores. Chromophores responsible for absorbance consist of conjugated double bonds and unbonded electrons like those associated with oxygen, sulphur and halogen atoms.

Kang et al. (2002) and Weishaar et al. (2003) reported that the different wavelengths of absorption have many useful applications in characterizing the aromatic carbon content, aromaticity and molecular weight of the dissolved organic matter (DOM). For example, UV absorbance at the 280 nm wavelength has been demonstrated to be a useful indicator of the aromaticity of a sample’s structure (Nanny and Ratasuk, 2002; Kang et al., 2002). The ratio of the absorbance at 465 nm and 665 nm (E_4/E_6 ratio) has also been used to provide an indication of the molecular size of the DOM. High molecular weight fractions, which have high carbon contents and relatively low numbers of oxygen and carboxyl groups, are associated with relatively low E_4/E_6 ratio values. Weishaar et al. (2003) applied the specific UV absorbance (SUVA), defined as the ratio of UV absorbance at 254 nm wavelength to DOC (UV_{254}/DOC) to estimate the dissolved aromatic carbon content. It was shown that SUVA was strongly correlated ($R^2 = 0.97$) with percent aromaticity determined by ^{13}C NMR for several organic matter isolates from different aquatic environments. Imai et al. (2002), Musikavong and Wattanachira (2007) and Saadi et al. (2006) also reported SUVA to be an important parameter in wastewater treatment plants.

UV absorption spectroscopy has also been used to measure the water quality parameters (Matsche and Strumworher, 1996; Chevalier et al., 2002; Chevakidagarn, 2005). Different wavelengths have been shown to correlate with BOD, COD and TOC. For example, Brookman (1997) investigated the absorbance at 280 nm for slurry and farm effluents and found that it could be useful for the rapid estimation of BOD. Chevalier et al. (2002) and Chevakidagarn (2005) reported that UV absorbance at 220 and 260 nm wavelengths correlated well with BOD. UV absorbance at 254 nm wavelength has been found by most authors to provide a close correlation with COD and DOC (Bari and Farooq, 1984; James et al., 1985; Matsche and Strumworher, 1996), and suggested UV absorbance at 254 nm as an excellent surrogate parameter for estimating the aromaticity of DOM.

2.8.2 Fluorescence spectroscopy

Fluorescence spectroscopy is a type of electromagnetic spectroscopy which analyses fluorescence from a sample. Fluorescence occurs when a loosely held electron in an atom or a molecule is excited to a higher energy level by the absorption of light and loses energy as the electron returns to its original energy level. Some energy within the molecules is lost through heat or vibration so that emitted energy is less than the exciting energy; i.e., the emission wavelength is always longer than the excitation wavelength.

Aromatic organic compounds are the principal fluorescence centres in organic matter because they have consecutive conjugated double or triple bonds (i.e. double or triple bonds separated by a single bond). Senesi et al. (1991) reported that the content of substituent groups such as carboxyls and carbonyls (having double bonds) also increases excitation and emission wavelengths. Molecules containing oxygen or nitrogen atoms, having lone electron pairs also enhance fluorescence (Senesi et al., 1991). Humic substances (humic and fulvic type molecules), the essential building block of recalcitrant organic compounds (Senesi et al., 1991; Yamashita and Tanoue, 2003) (described in section 2.6) are the principal fluorescent organic matter because these molecules contain aromatic compounds, and a high content of carboxylic groups, polycondensed aromatic and conjugated structures. Additionally, proteinaceous material exhibits fluorescence derived from the presence of aromatic amino acids such as phenylalanine, tryptophan and tyrosine (Coble, 1996).

Lackowicz (1999) reported that the wavelength at which the excitation and emission occurs is specific to particular molecules. For example, fluorescence peaks at 270-280 nm excitation and 305-312 nm and 340-370 nm emission can be ascribed to protein-like substances whereas peaks at 230-260 nm and 300-370 nm excitation and 400-470 nm emission can be considered as humic substances (Baker, 2002; Baker and Curry, 2004). In recent years Excitation-Emission-Matrix (EEM) fluorescence spectroscopy has become an increasingly used technique. It is based on the principle that excitation and emission wavelengths and fluorescence intensity can be scanned over a range of wavelengths synchronously and plotted on a single chart, developing a ‘map’ of optical space. Figure 2.2 presents a fluorescence 3D EEM for a hypothetical leachate sample. Fluorescence peaks may vary in intensity relative to each other depending on the organic matter source, whereas overall fluorescence intensity variations will reflect changes in concentration.

The important factor in the use of fluorescence spectroscopy is the effect of inner-filtering often referred to as re-absorption (Larsson, 2007). Re-absorption happens because another molecule or part of a macromolecule absorbs at the wavelengths at which fluorophore emits radiation. Another inner filtering effect (IFE) occurs when using concentrated solutions. Yang and Zhang (1995) defined inner filtering as a shift to longer emission wavelengths (red shift) due to higher concentrations of fluorophores in the solution and a shift to shorter emission wavelengths (blue shift) due to decreasing solution concentrations. Lackowicz (1999) reported that this IFE is important as it can reduce the fluorescence intensity by 5%. This mechanism is different from quenching, although quenching reduces fluorescence intensity as well. Lackowicz (1999) defined quenching as the process by which fluorescence is reduced because the excited molecules lose their energy via other processes, such as interaction with other molecules rather than emitting light. Various suggestions have been made about the appropriate correction for inner filtering effect (IFE) (Senesi, 1990, McKnight et al., 2001). Senesi (1990) suggested dilution as the easiest method to reduce IFE. Zsolnay et al. (1999) and Cox et al. (2000) stated that reducing the absorbance values of the sample <0.1 at 340 nm could avoid IFE. However, Tucker et al. (1992), Lackowicz (1999) and McKnight et al. (2001) applied a mathematical correction to fluorescence data to eliminate the effect of inner filtering. These methods are based on the measured absorbance of the sample. Since dilution is not always possible, and corrections based on absorbance are primarily based on the use of a separate absorbance instrument, neither of these solutions is optimal. In this case, Larsson et al. (2007) proposed a mathematical correction procedure based on the intensity of Raman scatter from water. This procedure was found to reduce the error after correction by up to 50% in comparison with Lackowicz (1999) absorbance correction procedure. Furthermore, it does not require the use of a separate absorbance measurement, and it is applicable to on-line and in situ EEM recordings, where the IFE would otherwise cause problems.

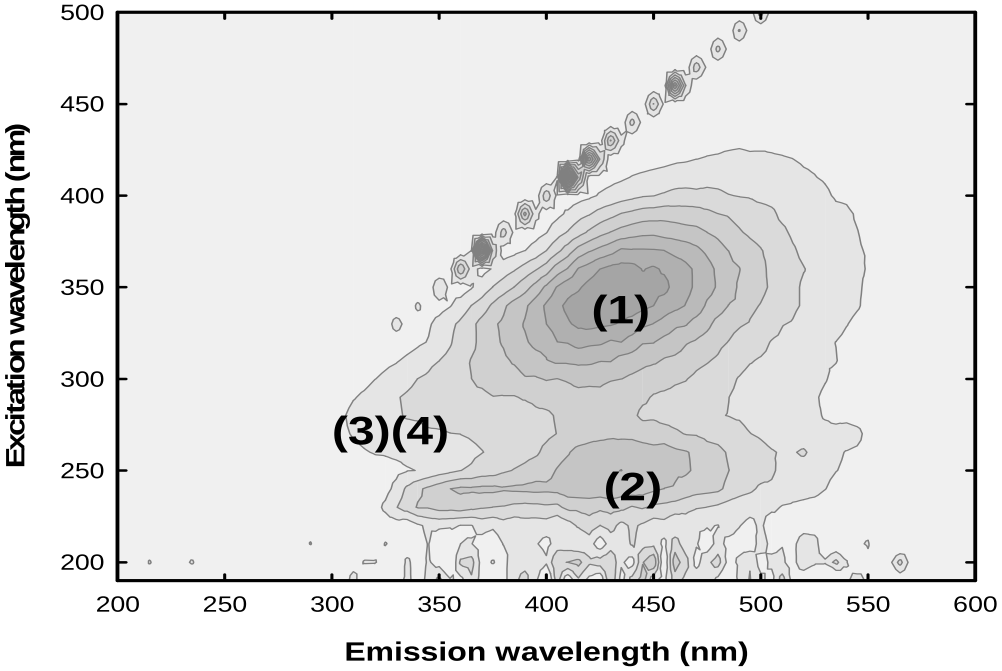


Figure 2.2 Fluorescence EEM for a hypothetical leachate sample to show all possible fluorescence centres (1) Fulvic-like (at 320-370 excitation and 400-470 emission) (2) Humic-like (at 230-260 and 300-370 excitation and 400-470 emission) (3) Tyrosine-like (at 270-280 excitation and 305-312 emission) and (4) Tryptophan-like (at 270-280 excitation and 340-380 emission) (Baker, 2002; Baker and Curry, 2004).

Fluorescence intensity depends on a number of environmental factors (Hudson et al., 2007). For example, fluorescence intensity is highly sensitive to pH. Huatala et al. (2000), Westerhoff et al. (2001) and Patel-Sorrentino et al. (2002) found an increase of fluorescence intensity (10-40%) in response to an increase in pH. Changes in observed fluorescence intensity are a result of conformational changes in the humic and fulvic molecules (Westerhoff et al., 2001) exposing or hiding fluorescent parts of the molecule. This phenomenon has been related to the fact that at high pH the macromolecule has a linear structure, and at low pH these structures contract. Patel-Sorrentino et al. (2002) has suggested that at low pH fluorophores may be situated within the coiled structures and are masked by non-fluorescent components and accordingly do not contribute to the fluorescence intensity. To overcome this, Her et al. (2003) suggested that all samples should be standardised at pH 7.0. Blaser et al. (1999), Sharpless and McGown (1999), Esteves da Silva et al. (1998), Elkins and Nelson (2001) and Fu et al. (2007) reported that fluorescence intensity depends on the presence of metal ions (aluminium, iron, copper, lead, nickel) through the formation of insoluble metal ion complexes. For example, Reynolds and Ahmad (1995) showed that aluminium and copper caused fluorescence intensity to be quenched by up to 40%. Temperature is also an important factor in fluorescence properties (Baker, 2005). Vodacek and Philpot (1987) stated that fluorescence is inversely related to temperature due to increased collisional quenching at higher temperatures. Conventionally, the temperature is held constant at 20°C during fluorescence analysis to avoid any interference from thermal quenching.

The fluorescence spectroscopic technique has been widely used in marine and estuarine waters, freshwater and wastewater to characterise DOM and to fingerprint the organic pollutants. The majority of fluorescence research has been carried out in marine waters to characterise the fluorescence properties of DOM. Coble (1996) examined the variability in fluorescence of natural DOM, attempting to distinguish between humic substances from terrestrial and marine sources. Several studies also applied fluorescence spectroscopy as a tool to determine the biological activity and protein fluorescence in marine environments (Determann et al., 1998; Parlanti et al., 2000; Yamashita and Tanoue, 2003; Jaffe et al., 2004). It was found that protein-like fluorescence (Tyrosine and Tryptophan-like) in marine and estuarine waters derived directly from biological activity (bacterial growth and death) (Parlanti et al., 2000; Yamashita and Tanoue, 2003).

However, the study of the DOM fluorescence in freshwater is not yet as widespread as that in marine science. Freshwater fluorescence has been investigated with an emphasis on spatial variation (Baker and Spencer, 2004; Fu et al., 2007). Baker and Spencer (2004) found that tryptophan-like fluorescence increased in river downstream with the increased anthropogenic input and urbanization. Similar results were observed in an urban river by Fu et al. (2007) and on a catchment scale by Baker et al. (2003). Baker (2002) and Baker et al. (2003) also observed that tryptophan-like fluorescence varied seasonally and was intense in summer. The authors attributed this to be due to reduced base flow and low dilution.

A number of studies have applied fluorescence EEM to characterize wastewater DOM in treatment processes (Reynolds and Ahmad, 1997; Reynolds, 2002; Westerhoff et al., 2001 and Her et al., 2003; Cammack et al., 2004; Elliot et al., 2006). These studies identified tryptophan-like fluorescence as being most likely to relate to the biodegradable fraction of wastewater, with a 90% reduction across a treatment process. Fluorescence EEM has been used to characterise the DOM in sewage impacted rivers (Baker, 2001), groundwater (Baker and Lamont-Black, 2001), wetland (Maie et al., 2007), lake water (Mostofa et al., 2005), sea ice (Stedmon et al., 2007) and soil (Ohno and Bro, 2007). The correlation between fluorescence intensity (FI) and chemical and biological water quality monitoring parameters such as BOD, COD and TOC (DOC) has been studied in various fields (urban river, Baker, 2002; Mostafa et al., 2005; Fu et al., 2007; river, wetland, spring, pond and sewage, Cumberland and Baker, 2007; landfill leachate, Baker and Curry, 2004). Additionally a number of studies have been carried out to track and characterise the presence of anthropogenic compounds by fluorescence signature in the freshwater system that include Fluorescent Whitening Agents (FWAs) from tissue mills and laundry products (Baker, 2002); a mixture of fluorescent xenobiotic organic matter (XOM), naphthalene from landfill leachate (Baker and Curry, 2004); material from agricultural effluent (Baker, 2002).

The above studies (section 2.7.1 and 2.7.2) suggest that UV and fluorescence spectroscopy are adaptable tools that might have potential for application in landfill leachate research. The major advantages are that these techniques are rapid (1 min per sample), non-destructive and do not require chemicals or sample preparation (Baker, 2001). Despite these advantages, the use of UV and fluorescence spectroscopy in characterising recalcitrant compounds in leachates is rare. A limited number of studies are reported. For example, following Baker and Curry's (2004) report of fluorescence properties of leachates, Zheng et al. (2007) and Ham et al. (2008) used this technique to detect polycyclic aromatic hydrocarbon (PAHs), phthalates (PAEs) and polychlorinated biphynyls (PCB) in landfill leachates. Similarly the technique has been applied for characterising leachates from landfills of different ages (Kang et al., 2002; Huo et al., 2008), and different treatment process (Huo et al., 2008). However, its use to detect the change in compositions of recalcitrant organic compounds with time and waste biodegradation has not been reported. In addition, UV and fluorescence spectroscopy allow differentiation between biodegradable and recalcitrant compounds as well as fingerprinting the other organic pollutants in leachates.

2.9 Summary

In this chapter, recalcitrant organic compounds in leachates and its removal by various leachate treatment processes were reviewed. It was found that a significant amount of recalcitrant organic compounds remain after the widely applied economically viable biological treatment processes. Applications and limitations of conventional leachate characterisation methods like BOD, COD and TOC were also discussed. These techniques usually require considerable sample preparation and cannot provide detailed compositional analyses of recalcitrant organic compounds. Available methods for investigating constituent organics of recalcitrant compounds were reviewed. It was found that degradation methods for compositional analysis have disadvantages such as unknown denaturing reactions, transformations and substantial DOC lost. Although spectroscopic methods FTIR and NMR are interesting, an unambiguous estimation of the structure of constituent organics often requires prior separation of identifiable sub-compounds using degradation. As a result, these techniques cannot be accepted as rapid and economic techniques for onsite compositional analysis of recalcitrant compounds in leachates. In search of an economically viable technique, the application of UV and fluorescence spectroscopy to marine and estuarine waters, freshwater, and wastewaters were reviewed. These studies imply that UV and fluorescence spectroscopy have potential for effective monitoring of recalcitrant organic compounds in leachates which could be applied to landfill leachate management system to assist and enhance routinely used characterisation methods. In the subsequent chapters, these techniques are used for characterisation of leachates to perform a feasibility study on the use of UV and fluorescence spectroscopy.

Chapter 3

Characterisation of wastes and leachates

3.1 Introduction

This chapter presents the detailed experimental procedures used to research the nature of the recalcitrant organic compounds in leachates. Two laboratory experiments were carried out in this regard. In one experiment, a laboratory scale aerobic biological treatment process was carried out on leachate samples collected from two UK MSW landfills, Pitsea and Rainham in Essex. This experiment provides information on the composition of recalcitrant organic compounds in various types of landfill leachates at different stages of treatment process. In the second experiment, Biochemical Methane Potential (BMP) tests were carried out on four types of solid waste samples (fresh waste, composed waste, newspaper waste and synthetic waste) to evaluate the effect of the composition of wastes on the recalcitrant organic compounds in the leachates generated during anaerobic biodegradation of the waste components. These two experiments aim to fulfil the first research objective of understanding the nature of the recalcitrant organic compounds in leachates; particularly how they are removed in the course of a biological treatment processes as well as how the concentration changes during anaerobic biodegradation of waste components. Finally the application of potentially useful techniques; UV absorption and fluorescence spectroscopy for investigating the nature of recalcitrant materials in leachates is presented. This aims to fulfil the second research objective on the basic feasibility study of the application of UV absorption and fluorescence spectroscopy for a rapid and an economical characterisation of the organic material in landfill leachates.

3.2 Aerobic biological leachate treatment

3.2.1 Materials (Landfill sampling sites and leachate composition)

Four untreated and two treated leachate samples were collected from two MSW landfills, Pitsea and Rainham, to investigate their properties. The leachates were characterized using a variety of methods (section 3.4). The characteristics of the landfill sites and the collected leachate samples, and details of the treatment carried out on the leachate samples, are summarised in Table 3.1. Both sites have been in operation for over 50 years. The Pitsea landfill, located 30 miles east of London, has received mainly MSW with commercial, industrial, hazardous and non-hazardous liquid wastes. The Rainham landfill, also located east of London on the north bank of the River Thames, contains old phases that have accepted MSW and hazardous industrial wastes, and more recent phases that contain predominantly MSW.

Table 3.1: Characteristics of landfills and leachate samples (Information provided by landfill operators**)**

Sample	Label	Nature of waste from which leachate derived	Nature of treatment
1	Pitsea (LTP)	**Landfill** Pitsea ▪ Mixed MSW, commercial, industrial and hazardous wastes, with hazardous liquid wastes until 2002, non hazardous liquid waste thereafter, wastes range in age from 0 to 80 years	Leachate was passed though a treatment plant consisting of aerobic rotating biological contactors
2	Pitsea (P4)	**Landfill** Pitsea**, phase 4** ▪ Predominantly MSW waste, with some industrial waste, with liquid non-hazardous wastes, wastes less than 4 years old ▪ The phases not be hydraulically isolated from each other. Large scale leachate recirculation for many years, significant potential of mixing of leachates from different areas.	Untreated
3	Rainham (LTP)	**Landfill** Rainham**, yellow phase** ▪ Predominantly MSW, some hazardous industrial wastes, over-tipped by recent non hazardous MSW, waste ~30-60 years old. ▪ The phases not be hydraulically isolated from each other, less potential of mixing of leachates from different areas.	Untreated
4	Rainham (FE)	**Landfill** Rainham ▪ Sample is effluent from leachate treatment plant, treating “red” and “yellow” phase leachate	Treatment primarily consists of oil separation, air stripping and settlement of iron sludge, followed by activated sludge aerobic treatment
5	Rainham (P2)	**Landfill** Rainham**, phase 2** ▪ Predominantly domestic MSW and non hazardous industrial wastes, waste 0-20 years old. ▪ The phases not be hydraulically isolated from each other, less potential of mixing of leachates from different areas.	Untreated
6	Rainham (LTP Haz)	**Landfill** Rainham**, red hazardous** ▪ Predominantly MSW, some hazardous industrial wastes. ▪ Elevated concentrations of chlorinated aliphatic and aromatic organic compounds than Rainham (LTP), waste ~30-60 years old. ▪ The phases not be hydraulically isolated from each other, less potential of mixing of leachates from different areas.	Untreated

3.2.2 Experimental methods

Aerobic biodegradation experiments on the four untreated and two treated leachate samples were carried out in 1 litre batch glass reactors over a period of 30 days. Air was supplied by an *AQUATEC* aquarium pump through 5 mm diameter poly-vinyl chloride (PVC) tubing via a 3 cm long airstone. The air streams into the reactors were maintained at a flow rate of 100 ml/min. 500 ml of diluted leachate samples (20 ml leachate samples: 480 ml BOD water) were in contact with air for 30 days at 20°C (The recipe for BOD water is given in section 3.2.3). 15 ml samples were taken on the 2nd, 6th, 10th, 15th, 20th, 25th, and 30th days of the treatment, filtered through a 25 mm micro glassfibres filter *(Fisher Brand, MF 200),* stored in the freezer and treated according to the scheme shown in figure 3.1. The results were corrected by multiplying by the dilution factor 25.

3.2.3 Preparation of BOD water

Leachate contains more oxygen demanding materials than the amount of dissolved oxygen available in the air saturated water. Therefore, it is essential to dilute the sample before incubation to bring the oxygen demand and supply into the appropriate balance and hence BOD water is used. 1 ml of each of the following four stock solutions was added to 1 litre distilled water to prepare the BOD water (APHA, 1998).

Reagents

1. Phosphate buffer solution: 8.5g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4.7H_2O$ and 1.7 g NH_4Cl were dissolved in about 500 ml distilled water and diluted to 1 litre. The pH was 7.2.

2. Magnesium sulphate solution*:* 22.5 g, $MgSO_4.7H_2O$ was dissolved in distilled water and diluted to 1 litre.

3. Calcium chloride solution*:* 27.5 g, $CaCl_2$ was dissolved in distilled water and diluted to 1 litre.

4. Ferric chloride solution*:* 0.25 g, $FeCl_3$ was dissolved in distilled water and diluted to 1 litre.

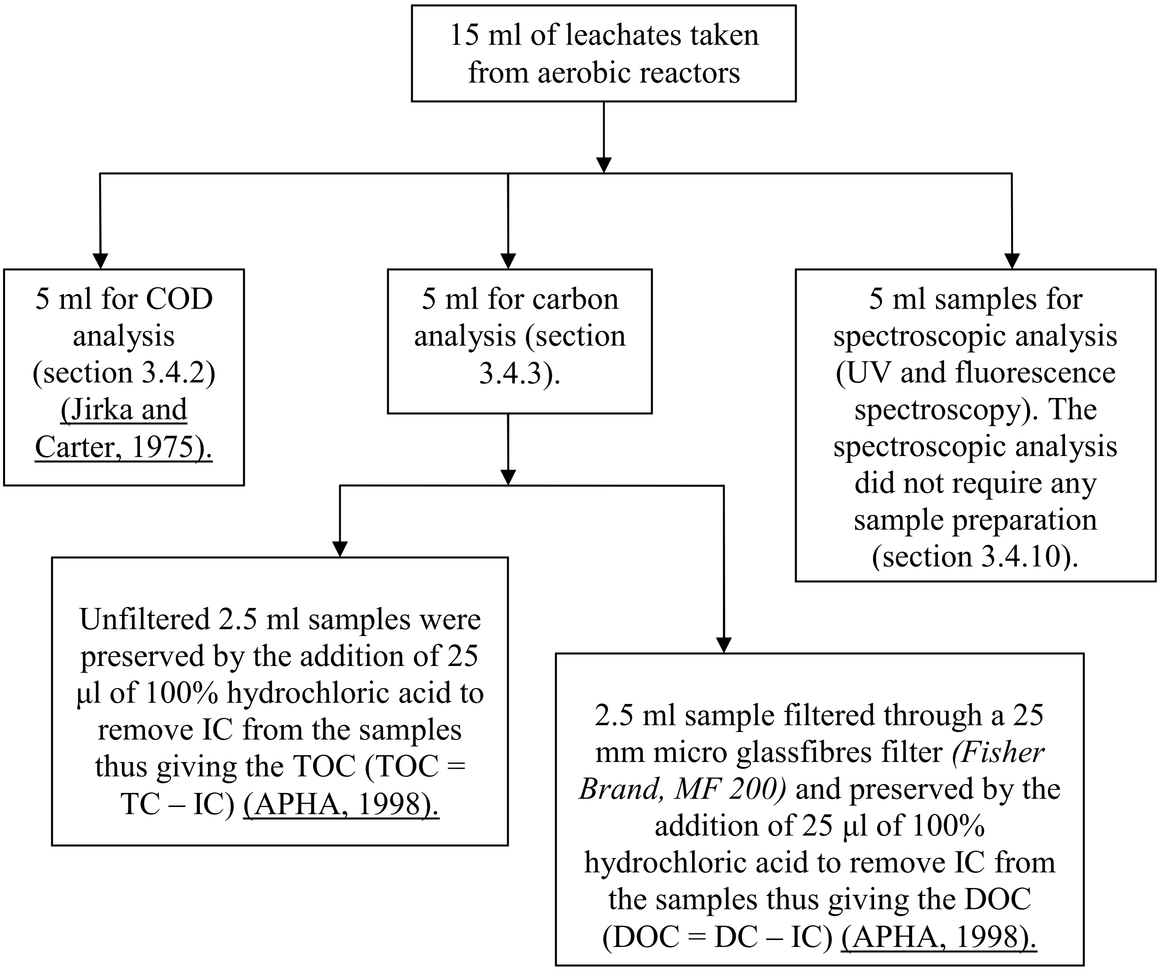


Figure 3.1 Analyses carried out on the leachates taken from aerobic reactors

3.3 Biochemical Methane Potential (BMP) test

Biochemical Methane Potential (BMP) tests were carried out to assess the variability in the anaerobic biodegradability of the component solid materials. There are several techniques for BMP testing of solid wastes, although all generally involve the incubation of a small representative waste sample under controlled anaerobic conditions (usually mesophilic at $30^{o}C$). However, the precise procedures in terms of pre-treatment of the samples, the inoculum, gas measurement techniques and incubation vary significantly among the published methods (e.g. Harries et al., 2001; Heerenklage and Stegmann, 2001;

Hansen et al., 2004). In this study, the BMP tests were carried out according to the procedures described by Zheng et al. (2007) and Ivanova et al. (2008).

3.3.1 Material (Pre-test waste sample preparation procedure)

In this experiment, characterization of four types of wastes was undertaken, FW- fresh waste, CW- composted waste, NW- newspaper waste and SW- synthetic waste (Table 3.2).

The most common method for characterizing a waste is to separate the waste into a number of different components and determine the percentage of each component. Fresh waste samples were supplied by the Otterbourne waste transfer station operated by Veolia Hampshire Ltd (Otterbourne, Winchester, Hampshire, UK), after separation into different components such as food, grass, newspapers and removal of the plastics and non-biodegradable components such as nappies, textiles, electrical appliances and construction material. The sorting analysis result of the fresh waste samples was supplied by the Otterbourne waste transfer station and is presented in Table 3.3. A sub-sample (20 kg) of the fresh waste was composted aerobically in the laboratory for 42 days to produce a sample of composted waste (CW) which was described in Zheng et al. (2007). Synthetic waste was prepared in the laboratory by mixing food, grass and newspapers wastes according to the composition of the supplied fresh waste as described in Table 3.3.

100 g of weighted sample of the fresh waste, composted waste, newspaper waste and synthetic waste were ground to a fine powder and analysed for lignin, cellulose and hemi-cellulose (section 3.4.8), TC and total nitrogen (TN) (section 3.4.7). For each sample triplicate experiments were carried out.

3.3.2 Experimental procedure

BMP tests were carried out on each of the four different types of waste samples (FW, CW, NW and SW) in twelve 1 litre plastic Nalgene bottles (three bottles for each waste sample). The BMP apparatus is shown in figure 3.2. 100 g of oven dried waste sample was placed in each reactor bottle. Individual reactor bottles were opened periodically in an anaerobic cabinet under a nitrogen atmosphere (it is acknowledged that replicate reactors would have provided a better statistical data set) to observe the compositional changes of leachates taken at different stages of the component waste biodegradation with respect to time of incubation. Solid compositions were determined at the beginning and at the end of the experimental period for each of the bottled wastes. Gas volumes were measured and samples of gas were taken at the end of the experiment to analyse methane (CH_4) and carbon dioxide (CO_2). Leachate sampling is outlined in Table 3.4.

Table 3.2 Types of waste samples

Waste Reference	**Waste description**
Fresh waste (FW)	Fresh waste sample obtained from Otterbourne waste transfer station operated by Veolia Hampshire Ltd (Otterbourne, Winchester, Hampshire, UK).
Composted waste (CW)	Composted waste obtained from the composting of fresh waste for 42 days in the laboratory (Zheng et al., 2007).
Newspaper waste (NW)	Newspaper waste.
Synthetic waste (SW)	Synthetic waste was prepared in the laboratory same to the composition as the fresh waste.

Table 3.3 Breakdown of waste composition for fresh waste samples

Component	**Dry mass (g)**	**% by dry mass**
Newspaper/card board	124.15	34.39
Food	105.05	29.10
Grass	131.80	36.51
Total	**361.00**	**100.00**

Figure 3.2 BMP test assay apparatus

Table 3.4 Frequency of leachate analysis

BMP reference	**Days into experiment**	**Leachate sampling frequency (Day)**
BMP (FW, CW, NW and SW sample)	0-20	Every 2nd day
	20-70	30, 50, 70
	70-150	80, 90, 100, 120, 140, 150

To accelerate the degradation of the waste by anaerobic bacteria, 700 ml of a laboratory prepared methanogenic medium containing mineral nutrients (nitrogen, phosphorous, and sulphur), trace elements and anaerobic digested sewage sludge (10% by volume) derived from an anaerobic digester at Millbrook Sewage Works (Southern Water, UK) was added to each BMP reactor to act as a seed inoculum. The medium was adapted from Florencio et al. (1995) and is presented in Table 3.5. The final pH of the medium was adjusted to pH 7.83 by adding 2M NaOH.

Table 3.5: Recipe of the methanogenic mineral medium (Florencio et al., 1995)

Reagents	Concentration (mg/l)	Reagents	Concentration (mg/l)
NH_4Cl	280.00	$CuCl_2.2H_2O$	0.04
$K_2HPO_4.3H_2O$	330.00	$(NH_4)_6MoO_{24}.4H_2O$	0.05
$MgSO_4.7H_2O$	100.00	$Na_2SeO_3.5H_2O$	0.16
$CaCl_2.2H_2O$	10.00	$CoCl_2.6H_2O$	2.00
$FeCl_2.4H_2O$	2.00	$AlCl_3.6H_2O$	0.09
H_3BO_3	0.05	$NiCl_2.6H_2O$	0.14
$ZnCl_2$	0.05	EDTA	1.00
$MnCl_2.4H_2O$	0.50		

The mineral medium was kept under a nitrogen atmosphere for one day to remove oxygen. The waste samples were then mixed with methanogenic medium and inoculum and sealed under anaerobic conditions before being placed in a water bath at 30°C and incubated to promote mesophilic methanogenic conditions. No mechanical mixing of the waste took place during the test.

In this study the BMP tests were carried out over a period of 150 days although Harries et al. (2001) showed that virtually all the gas was produced from a similar waste samples in 90 days.

Biogas production was measured by collecting the gas produced from each bottle in an inverted glass burette containing water acidified with HCl to a pH of 2.0. The acidwater was displaced as the gas accumulated within the burette allowing the gas volume to be measured. Acidwater was used to prevent CO_2 dissolution. Three control (blank) reactors containing 700 ml of the mineral nutrient/trace element sewage sludge mix were used to determine the volume of gas produced from the inoculum alone. The measured volume of biogas produced was corrected to standard temperature and pressure (STP) using the ambient temperature and pressure measurements (Eq.3.1).

$$R = R_0 (\frac{P_a}{101.3}) x (\frac{273.2}{273.2 + T}) \qquad \text{(Eq.3.1)}$$

where, *R* is the corrected reading, R_0 is the uncorrected reading, 101.3 is the atmospheric pressure at sea level in kPa, P_a is the measured atmospheric pressure in the laboratory in kPa, 273.2 is the temperature in Kelvin at 0^oC, and *T* is the reference temperature in the laboratory in ^oC.

3.3.3 Leachate sampling

The BMP test reactors were opened sequentially under a nitrogen atmosphere at various times to observe compositional changes in the leachates. 20 ml leachate samples were taken from each bottle periodically, half of which was then filtered through 1.2 µm Whatman GF/C filters. Equivalent volumes of the methanogenic mineral medium were then added to the bottles to keep the volume of the liquid unchanged. Unfiltered and filtered leachate samples were diluted and analysed as shown in figure 3.3.

3.3.4 Solid waste sampling

The composition of the four types of solid waste samples was determined by elemental and fibre analysis at the beginning and at the end of the experiment to assess changes due to biodegradation. The procedure for the preparation and analysis of the solid waste samples is shown in figure 3.4.

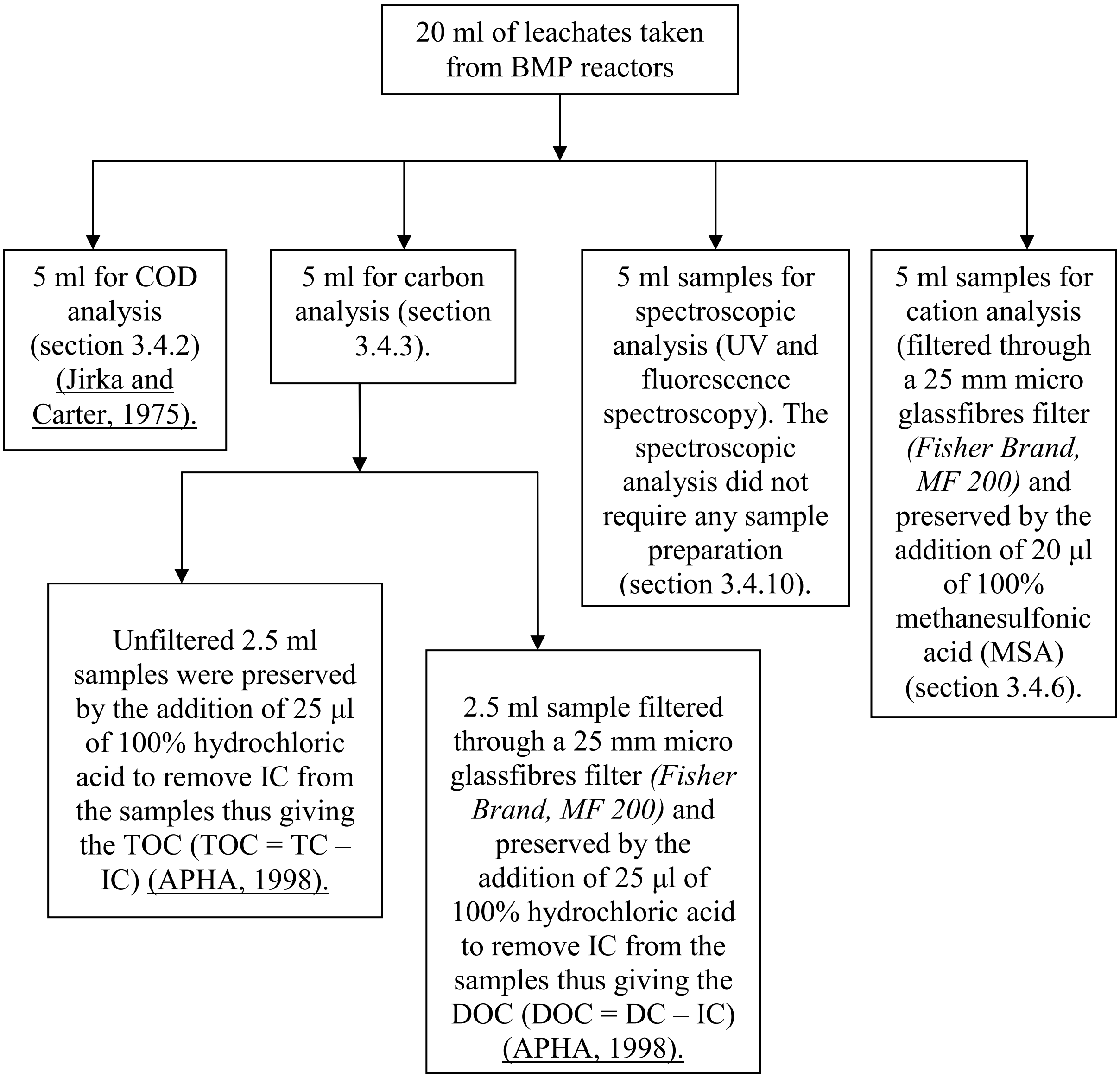


Figure 3.3 Analyses carried out on the leachates taken from BMP reactors

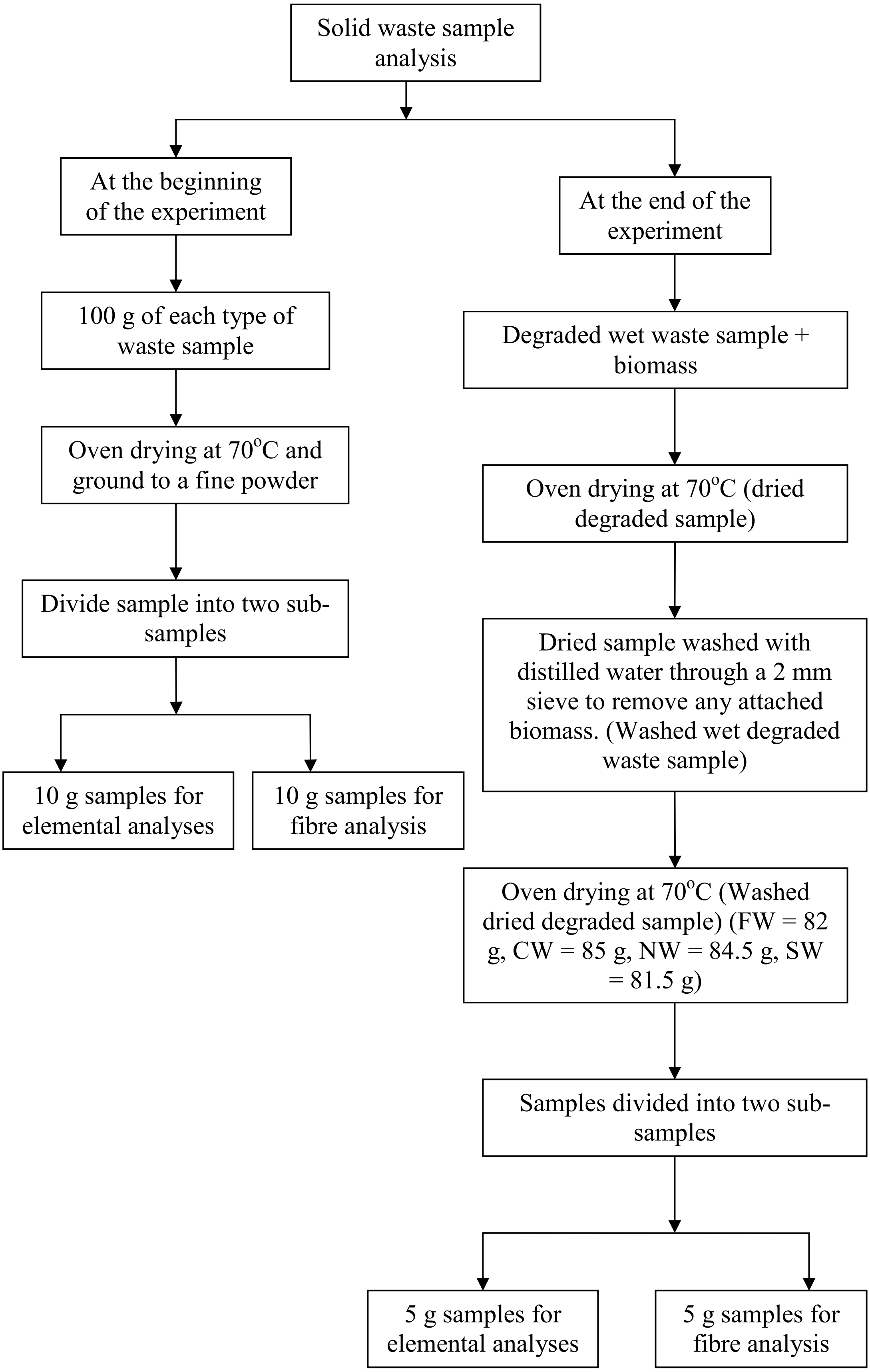


Figure 3.4 Analyses carried out on the solid samples from BMP reactors (Ivanova et al., 2008)

3.4 Analytical measurements

3.4.1 Biochemical oxygen demand (BOD) analysis

The BOD_{30} of the landfill leachate samples were analysed using the WTW Oxi-Top control system. The system uses respirometric measurement whereby if O_2 is consumed in a closed vessel at constant temperature, a negative pressure develops as the carbon dioxide (CO_2) produced is absorbed by the potassium hydroxide (KOH) pellets at the top of the vessel. The ***OxiTop*** measuring head measures and stores this pressure for the whole duration of the BOD test. 22.7 ml of landfill leachate samples were used for BOD test.

The BOD is calculated using Equation 3.2

$$BOD = \frac{M(O_2)}{R.T_m}.(\frac{V_t - V_1}{V_1} + \alpha \frac{T_m}{T_0}).\Delta p(O_2) \qquad \text{(Eq. 3.2)}$$

Where,

$M(O_2)$ = molecular weight (32000 mg/mol)

R = gas constant (83.144 mbar/mol-K)

T_0 = reference temperature (273.15 K)

T_m = measuring temperature

V_t = bottle volume (ml)

V_1 = sample volume (ml)

α = Bunsen absorption coefficient (0.03103)

$\Delta p(O_2)$ = difference of the oxygen partial pressure (mbar)

3.4.2 Chemical oxygen demand (COD) analysis

COD measurements of the leachate samples taken from the aerobic biodegradation reactor and also from the BMP reactors (figures 3.1 and 3.3) were carried out using the micro-digestion technique (Jirka and Carter, 1975). The Standing Committee of Analysis (SCA) standard for COD analysis was not used in this study because the SCA method was limited by a maximum detection limit up to 400 mg/l of COD while in this study considerably high COD values were presumed. The COD determination provides a measure of the oxygen equivalent of that portion of the organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant.

5 ml of sample volumes (figures 3.1 and 3.3) filtered through a 25 mm glass microfibre filter *(Fisher Brand, MF 200)* were refluxed with known amounts of potassium dichromate and sulphuric acid (COD reagent), and the excess dichromate was titrated with ferrous ammonium sulphate (FAS). The amount of oxidizable organic matter, measured as oxygen equivalent, was proportional to the potassium dichromate consumed.

COD is calculated as follows:

$$COD = 8000 * (V_B - V_S) * M_{FAS} * D_f/\text{sample volume} \qquad \text{(Eq. 3.3)}$$

Where,

V_B = volume of FAS used in titrating the appropriate blanks (ml)
V_S = volume of FAS used in titrating the sample (ml)
M_{FAS} = molarity of FAS
D_f = dilution factor
8000 = milli-equivalent weight of oxygen x 1000 ml/L

Because COD measures the oxygen demand of organic compounds in a sample of leachate, it is important that no outside organic material be accidentally added to the sample to be measured. To control this, a blank sample was created by adding all reagents (e.g. acid and oxidizing agent) to a volume of distilled water. COD was measured for both the leachate sample and blank samples, and the two were compared. The COD in the original sample was subtracted from the COD for blank sample to ensure a true measurement of organic matter.

3.4.3 Carbon analysis

A high-temperature total organic carbon analyzer (Dohrman Rosemount DC-190, USA) was used to measure the leachate total carbon (TC), total inorganic carbon (TIC) and total organic carbon (TOC). The equipment contains a vertical quartz combustion tube packed with cobalt catalyst. Oxygen flows through it at a rate of 200 ml/min. The furnace was operated at 800°C. The manual injection mode was used for the leachate samples collected from the landfills at Pitsea and Rainham. The samples were analysed three times and the average value taken. The boat sampling mode was used due to the high concentration of suspended solids in the unfiltered leachate samples collected from the BMP test reactors.

The equipment utilized a single point calibration which was carried out each time prior an analysis. TC and TIC standard were prepared using Glycine (NH_2CH_2COOH) and sodium bicarbonate ($NaHCO_3$) respectively at a concentration level depending on the concentration range of the samples.

3.4.4 Solids content

Total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), and total dissolved solids (TDS) of landfill leachate samples were carried out according to the standard methods (APHA, 1998). TS contents were determined by oven drying at 105°C and VS by oven drying at 550°C. Again the Standing Committee of Analysis (SCA) standard for TS, VS, TSS, VSS, and TDS analyses was not used due to the uncertainty of the recommended filter size and manufacturer.

3.4.5 Anion analysis (Cl^-, NO_2^-, NO_3^-, PO_4^{-2}, SO_4^{-2})

5 ml landfill leachate samples were filtered through a 25 mm glass microfibres filter *(Fisher Brand, MF 200)* and the filtrate were immediately frozen. Anion analysis was carried out using a Dionex-500 ion chromatograph with an AS9 anion column in conjunction with an ASRS-1 anion suppressor (Dionex Ltd.). 25 µl volume injections were applied to the column using a Dionex AS-40 auto-sampler, incorporating a rheodyne valve. Detection was by a Dionex ED-40 electrochemical detector operating in conductivity mode. The eluent consisted of 8 mM sodium carbonate and 1 mM sodium bicarbonate pumped at a flow rate of 1.0 ml/min.

A calibration procedure was carried out prior to each analysis using anion stock solution prepared from a mixture of NaCl, $NaNO_2$, KNO_3, KH_2PO_4 and $MgSO_4.7H_2O$ with concentration levels of 50, 100, 300, 600, 800, 1000 µM.

3.4.6 Cation analysis (Na^+, NH_4^+, K^+, Mg^{+2}, Ca^{+2})

5 ml volumes of filtered leachate samples were used for the cation analysis. This was carried out using a Dionex-500 ion chromatograph with a CS12A cation column in

conjunction with a CSRS-II cation suppressor (Dionex Ltd.). The mobile phase was 20 mM methanesulfonic acid pumped at a rate of 1.0 ml/min.

A calibration procedure was carried out prior to each analysis using cation stock solution prepared from a mixture of NaCl, NH_4NO_3, KH_2PO_4, $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ with concentration levels of 50, 100, 300, 600, 800, 1000 μM.

In this study CEN standard for anion and cation analyses was not used because of the potential disadvantage of low detection limit for various elements.

3.4.7 Elemental analysis

The total carbon (TC) and total nitrogen (TN) content of the solid waste samples (FW, CW, NW and SW) at the beginning and at the end of biodegradation process were determined using a CE Instruments 1112 Flash Elemental Analyser. Prior to CHNS analysis, solid waste samples were pre-treated according to the procedure described in section 3.3.4 (figure 3.4). Dried samples were ground to fine powder using a Knifetec 1095 Sample Mill (Foss) and then weighed (2-5 mg) in tin containers. TC and TN contents of the samples were determined by dry combustion at 900^{o}C in an oxygen atmosphere with 140 ml/min helium carrier gas and Thermal Conductivity Detector (TCD) detection of gases produced. L-cysteine, Methionine and Sulphanilamide were used as a standards and Vanadium pentoxide was used as sample additive to help in oxidation.

3.4.8 Fibre analysis (cellulose, hemi-cellulose and lignin)

Fibre tests provide values for cellulose, hemi-cellulose and lignin content in the waste (Van Soest et al., 1991). The major methods used to determine fibre fractions are the Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Digestible Lignin (ADL) tests. The NDF test dissolves readily degradable material such as pectins, sugars, starch and fats and leaves behind cell wall components of plant material, cellulose, hemi-cellulose and lignin. The ADF test in contrast, digests less degradable hemi-cellulose and some proteins leaving a residue of cellulose, lignin and bound nitrogen. The ADL test is a measure of lignin (Van Soest et al., 1991).

The components determined by these tests can be summarised as follows:

NDF = Cellulose + Hemicellulose + Lignin + Mineral Ash (Eq. 3.4)

ADF = Cellulose + Lignin + Mineral Ash (Eq. 3.5)

ADL = Lignin + Mineral Ash (Eq. 3.6)

Fibre analysis on the dried solid waste samples (section 3.3.4) was carried out using the Foss Technology system FibreCap 2021/2023 (Kitcherside et al., 2000) at the beginning and at the end of the BMP experiment. Prior to analysis solid waste samples were pre-treated according to the procedure described in section 3.3.4 (figure 3.4). Dried samples were ground to a fine powder using a Knifetec 1095 Sample Mill (Foss) and different fibre fractions were analyzed several times. Less than 0.5 g of each sample was prepared by multiple measurements using ultra sensitive weighting machine which was then used in each capsule. The NDF test was carried out using chemical procedures as described by Van Soest et al. (1991), except that the sample was retained in the FibreCap capsule. This procedure uses α-amylase at two stages in the extraction process to improve the solubilization of starch. The ADF test involves digesting the waste samples using a cationic detergent (*Cetyltrimethylammoniumbromide*, 20 g/l) in 0.5M sulphuric acid for 1 hour after reaching boiling point. The ADL was assessed by further treating the non-dried and non-ashed residues from previously performed ADF tests (first-step digestion) with 72% sulphuric acid at room temperature for 4 hours to dissolve the cellulose, leaving lignin as the residue (Effland, 1977). The specific cell wall components (cellulose and hemi-cellulose) were determined using Eq. (3.7) and Eq. (3.8).

Cellulose and hemi-cellulose can be determined according to the following equations:

Cellulose = ADF – ADL (Eq. 3.7)

Hemi-cellulose = NDF – ADF (Eq. 3.8)

Lignin = ADL (Eq. 3.9)

3.4.9 Gas analysis

Gas samples were collected using hypodermic syringes which were inserted through the three way valves installed on the top of each BMP reactor. The samples were immediately analysed.

The composition of biogas (CH_4 and CO_2) at the end of the BMP experiment was determined by gas chromatograph (GC Varian 3800 gas chromatograph) equipped with a Thermal Conductivity Detector (TCD) and two columns (a Haysep C 80-100 mesh a Molecular Sieve 13 x 60-80 mesh, Analytical column, UK). Both columns were 1 meter long, 6 mm diameter and were operated isothermally at $50^{o}C$. TCD and injection temperature were $200^{o}C$ and $100^{o}C$ respectively. The injection volume was 0.25 ml. Carrier gas argon flows through it at a rate of 6 ml/min.

The calibration procedure was carried out prior to each analysis using a calibration mixture of CH_4 and CO_2 with concentration levels of 65% and 35% respectively.

3.4.10 Spectroscopic characterization of leachate DOC

5 ml volumes of leachate samples were used for spectroscopic analysis (figures 3.1 and 3.3). Spectroscopic analysis did not require any sample preparation except for the fact that leachate samples were adjusted to pH 7.0 using 2M NaOH (section 2.8.2) because fluorescence intensity is highly sensitive to pH change.

3.4.10.1 *UV*-visible spectroscopy

UV-visible absorption spectra of leachate samples were recorded on a Cecil Spectrophotometer using a 10 mm quartz cell. Distilled water was used as blank. Leachate samples were diluted until the absorbance value fell below 0.1/cm at 340 nm wavelength (Baker, 2005). Scan spectra of the solutions were obtained over a wavelength range of 200-800 nm. Absorbance at 254, 465 and 665 nm wavelengths was also measured (section 2.7.1). SUVA values (l/mg/cm) as a measure of the relative contents of aromatic structures in the overalls DOM were calculated as (UV_{254}/DOC) x 100 (Weishaar et al., 2003).

3.4.10.2 Fluorescence spectroscopy

Fluorescence Excitation-Emission-Matrix (EEMs) were measured in a standard 10 mm quartz cell using a Varian Cary Eclipse fluorescence spectrofluorometer equipped with a temperature controller to enable the measurement of EEMs at precisely controlled temperatures. The temperature throughout the study was held constant at 20^{o}C in order to avoid any interference from thermal quenching (Baker, 2005) (described in section 2.8.2**)**. Three dimensional EEMs were generated at excitation and emission slit widths of 5 nm band pass. All samples were scanned in the excitation wavelengths 190-800 nm in 10 nm steps and emission wavelengths 200-800 nm in 10 nm steps. The spectra of water blank was obtained in the same conditions and was subtracted from the original spectra of leachates to eliminate water Raman scatter peaks. Scan speed was 9600 nm/min. Samples containing high concentrations of leachate were diluted to required dilution as described in section 3.4.10.1 due to their high fluorescence intensity and 3D maps were built using Sigma-plot 10 software. Coordinates of the main noticeable peaks were established in these maps. Peak positions were not affected by this dilution (as tested for selected samples of this study), indicating that this also avoided any inner-filtering effects (Hudson et al., 2007).

EEMs are illustrated as the elliptical shape of the contours. The X and Y-axes represent the emission and excitation wavelength respectively. Contour lines are shown for each EEM spectrum to represent the fluorescence intensity. The total fluorescence intensity was calculated by the cumulative integration of the intensity versus emission wavelength data for any given excitation wavelength range corresponding to different types of materials. The final intensity value was corrected by multiplying by the dilution factor.

Emission spectra were recorded in emission scanning mode over the range 300-500 nm at excitation wavelengths 230-260 nm and 320-370 nm. Fluorescence spectra in emission scanning modes provided important aromatic structural information with the position of the fluorescence peaks of the different compounds. The total intensity was calculated by adding the intensities from the intensity versus emission wavelength data for every excitation wavelength.

3.5 Carbon mass balance estimate

Carbon mass balance estimates for each of the waste samples (FW, CW, NW and SW) at the end of the BMP tests were carried out using the formula given by Ivanova et al. (2008) (Eq. 3.10)

$$\sum m_{in} = \sum m_{out} + \sum m_{accumulated} \qquad \text{(Eq.3.10)}$$

This equation states that the sum of the masses flowing into a system should be equal to the sum of the masses flowing out of the system and the sum of the mass being accumulated within the system. The BMP test analysis had two input terms, i.e., the total carbon of the initial waste and of the mineral media; two output terms, i.e., the total carbon from the produced biogas and final leachate; and two measured accumulated terms, i.e., the carbon content in the degraded waste and in the Ca and Mg carbonate precipitates.

The carbon mass balance error can be estimated with the following equation (Ivanova et al., 2008).

Mass balance error = Expected value – Actual value, (g/Kg DM) (Eq.3.11)
Where,
Expected value = Total carbon of the initial waste + Total carbon of the initial mineral media, (g/Kg DM) and
Actual value = Total carbon from the produced biogas + Total carbon of the final leachate + Total carbon from degraded waste + Carbon precipitated as Ca and Mg carbonates ($CaCO_3$ and $MgCO_3$).

The error can also be expressed as a percentage of the expected value:
Mass balance error, % = (Expected value – Actual value)/ Expected value x 100

The carbon output produced in the reactors was calculated from the volume of the biogas produced in each reactor and the CH_4 and CO_2 concentrations measured in the collecting burette (Ivanova et al., 2008). Biogas production was measured at 30^oC. All biogas readings were standardized to gas at STP using Eq. 3.1.

The total mass values of leachate carbon were estimated using the volumes of the leachate in the reactors and the weight of the waste material from which the total carbon was sourced. The carbon from the precipitation of $CaCO_3$ and $MgCO_3$ was calculated from the changes in the concentrations of Ca^{2+} and Mg^{2+} at the beginning and at the end of the experiment, the volumes of the leachate in the reactors and the weight of the waste material (Ivanova et al., 2008).

Chapter 4

Aerobic biodegradation of landfill leachates

4.1 Introduction

This chapter investigates the nature of the recalcitrant organic compounds in leachates in the course of a biodegradation by several methods. Landfill leachates were collected from two UK MSW landfills, Pitsea and Rainham (section 3.2.1) and an aerobic biological treatment process was carried out (described in section 3.2). At these sites, a range of hazardous wastes have been co-disposed with municipal solid wastes and there is no hydraulic isolation between different phases. The collected samples might represent mixture of leachates generated from a diverse range of waste composition and age and hence, the effect of a biodegradation process on the evolution of recalcitrant compounds in these leachates merits investigation. As discussed in Chapter 2, among different degradation/treatment methods existing in the literature, biological processes are mostly used in the UK for an economically viable leachate treatment. Therefore, in this study an aerobic biological treatment process was chosen to investigate the evolution of recalcitrant organics which would also provide useful information about biological treatment of leachates in these two landfill sites. The aerobic biodegradation experiments on the collected leachate samples were carried out in glass reactors over a period of 30 days with an air supply arrangement. The detail experimental set up is discussed in Chapter 3 (section 3.2.2).

The investigation of the recalcitrant organics was carried out by established methods of COD and DOC measurements, and by the potential new techniques, UV and fluorescence spectroscopy. A combined analysis was carried out to investigate the overall biodegradability of different leachate samples and to investigate the proportion and individual biodegradation of several organic constituents of leachates in course of the biodegradation. As such, these investigations provide an insight into the recalcitrant organic compounds in these landfill leachates.

These investigations therefore aim to:

1) develop an understanding of the characteristics of the recalcitrant organic compounds in real leachates during aerobic biological treatment
2) study the possible use of UV and fluorescence spectroscopy for fingerprinting various organic compounds as well as for analysing the changes in the compositional characteristics of the recalcitrant organic compounds in the course of a treatment by assisting routinely used analytical methods

4.2 Initial characterisation of landfill leachate samples

The chemical compositions of four untreated and two treated leachate samples collected from Pitsea and Rainham landfills are summarized in Table 4.1.

4.2.1 pH values

Table 4.1 shows that the pH of the collected leachates was in the range 7.20-8.42. The pH of leachate usually increases with time due to decrease of the concentration of free volatile fatty acids (Chian and Dewalle, 1976; Pohland and Harper, 1985). The reported pH of acidogenic leachates ranges from 5.6 to 6.9 whereas the pH of methanogenic leachates is in the range 6.8-8.0 (Lo, 1996; Lu et al., 1985; McBean et al., 1995). The pH values for the untreated samples in this study therefore indicating a methanogenic state although the leachates here may come from a mixture of old and new wastes within the sites.

4.2.2 Organic contents of leachates

Table 4.1 shows that all of the untreated and treated leachate samples had BOD_{30} values in the range of 85-452 mg/l and COD values in the range of 850-4500 mg/l. As discussed in chapter 2, BOD/COD values are correlated with the age of the landfill. Cho et al. (2002), Tatsi et al. (2003), Lopez et al. (2004), Cecen and Aktas (2004) reported that leachates from young landfills are characterised by high BOD and COD concentrations with values ranged from 2300 to 25000 mg/l and 10540 to 70900 mg/l respectively, whereas in old landfill leachates BOD and COD values ranges from 62 to 800 mg/l and 1409 to 3460 mg/l respectively.

Table 4.1 Chemical composition of landfill leachate samples

Parameter (mg/l) (except pH)	**Sample 1 (treated)**	**Sample 2 (untreated)**	**Sample 3 (untreated)**	**Sample 4 (treated)**	**Sample 5 (untreated)**	**Sample 6 (untreated)**
	Pitsea (LTP)[a]	**Pitsea (P4)**	**Rainham (LTP)[a]**	**Rainham (FE)[b]**	**Rainham (P2)**	**Rainham (LTP HAZ)[c]**
pH	8.42	7.72	7.20	7.60	8.12	7.40
BOD$_{30}$[d]	282	85	113	226	452	282
COD	3300	4500	1100	850	3700	950
BOD$_{30}$/COD	0.09	0.02	0.10	0.27	0.12	0.30
TC	1026	2728	644	243	2117	854
TIC	351	1477	373	104	1422	778
TOC	675	1251	271	139	695	76
DOC	663	1191	232	125	680	64
TS	17190	32890	7170	8200	10870	7400
TVS	15480	18080	1120	1620	2140	920
TFS	1710	14810	6050	6580	8730	6480
TSS	475	170	167	230	170	150
VSS	100	80	125	110	40	50
TDS	16713	32710	7000	7830	10490	7220
Chloride (Cl$^-$)	675	680	701	242	729	759
Nitrate (NO$_3^-$)	0.63	0	1.40	1.0	4.30	5.0
Sodium (Na$^+$)	382	306	325	307	295	298
Ammonium (NH4$^+$)	284	315	343	325	325	341
Potassium (K$^+$)	565	461	481	449	456	474
Magnesium (Mg^{2+})	57	59	17	11	10	14
Calcium (Ca^{2+})	696	611	325	254	268	215

[a] Leachate Treatment Plant

[b] Final Effluent

[c] Hazardous

[d] 30 days BOD

Chian and DeWalle (1976), Chian (1977) and Harmsen (1983) also demonstrated that the leachates generated from old landfills consist mainly of high molecular weight recalcitrant organic compounds, which are correlated with low BOD and COD values. Thus the low BOD and COD values of the collected leachate samples in this study implying significant leaching from the old wastes in the sites and hence, considerable amount of recalcitrant organic compounds should be expected to be present. This can also be verified by the corresponding BOD/COD ratio which is commonly known as a measure of biodegradability (Lo, 1996; Chen et al., 1996). The BOD/COD ratios of the collected leachate samples ranged from 0.02 to 0.30. Studies reported in the literature indicate that leachates containing high molecular weight recalcitrant organic compounds had BOD/COD ratios in the range of 0.01-0.40 (Timur and Ozturk, 1999; Marttinen et al., 2002; Cho et al., 2002; Tatsi et al., 2003; Lopez et al., 2004; Cecen and Aktas, 2004).

Table 4.1 also shows that although Pitsea (P4) leachate had been collected from the phase where wastes were less than 4 years old (Table 3.1), it had a high pH and low values of BOD and a low BOD/COD ratio. This may indicate that methanogenic conditions may have been established at an early stage in this phase. The early establishment of methanogenic conditions in this phase may be attributed to a high amount of readily degradable organic waste and the high moisture content (Table 3.1) allowing fast dissolution of organic compounds and accelerating microbiological decomposition. This may also be due to the fact that older leachates were mixed in with leachates from this phase. The early establishment of methanogenic condition has also been reported in the literature (Lo, 1996; Kulikowska and Klimiuk, 2008). Kulikowska and Klimiuk (2008) showed that in landfills in Poland, methanogenic conditions had been established at an early stage with stable COD values of 610 mg/l after about 4 years.

The total carbon content of the untreated leachate Pitsea (P4) was relatively higher than the untreated leachates collected from Rainham implying that organic and inorganic compounds in the Pitsea landfill was considerably higher than the Rainham landfill. However, the treated leachates collected from each of these landfills generally showed lower total carbon content in comparison to the untreated leachates thereby indicating the effect of treatment.

4.2.3 Solid contents

The concentrations of total solids (TS), total volatile solids (TVS), total fixed solids (TFS), total suspended solids (TSS), volatile suspended solids (VSS) and total dissolved solids (TDS) are presented in Table 4.1. With the exception of TFS and VSS of Pitsea (LTP), the leachates from Pitsea generally had a higher solids content than the leachates from Rainham. In the Rainham leachates the TFS contents were higher than the TVS contents for all of the untreated and treated leachate samples, whereas for the Pitsea leachates the trend was reversed. This indicates that the leachates from Rainham contained higher levels of inorganic solids whereas those from Pitsea contained higher levels of organic solids. TSS, VSS and TDS contents in all of the untreated and treated leachate samples ranged from 150 to 475 mg/l, 40 to 125 mg/l and 7000 to 32710 mg/l respectively, which are significantly higher than the values reported in the literatures (Al-Yaqout and Hamodoa, 2003; Fan et al., 2006). This reflects a high degree of mineralization during active anaerobic decomposition of the waste in Pitsea and Rainham landfills (Al-Yaqout and Hamodoa, 2003).

4.2.4 Anions and Cations

The concentrations of chloride (Cl^-), NO_3- nitrogen, ammonia-nitrogen (NH_3-N), sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) are presented in Table 4.1. The values of ammoniacal-nitrogen (NH_3-N) observed in all of the untreated and treated leachate samples were at the high end of the concentration range reported in the literature (Chu, 1994; Marttinen et al., 2002; Silva et al., 2004). This might be due to the hydrolysis and fermentation of the nitrogenous fraction of biodegradable substrates (Carley and Mavinic, 1991). In comparison to ammoniacal-nitrogen (NH_3-N), low NO_3- nitrogen values were found for all of the leachates, indicating that the majority of the nitrogen was in the form of ammonia. The results also show that the values of Na^+, K^+, and Cl^- observed in all of these leachate samples were in the high concentration range (Fan et al., 2006; Chu et al., 1994; Al-Yaqout and Hamodoa, 2003). The high values of these salt contents are correlated with high conductivities and the high TDS values (section 4.2.3). The Mg^{+2} and Ca^{+2} contents of the Pitsea leachates were higher than those of Rainham leachates. This might be due to higher industrial wastes disposed in Pitsea landfill than in Rainham landfill (Table 3.1) (Kulikowska and Klimiuk, 2008).

The chemical compositions of leachates from these two UK landfills are compared with landfills in Taiwan, Hong Kong, Kuwait, USA, Germany, Turkey, Finland and Greece in Table 4.2 (Fan et al., 2006; Chu et al., 1994; Al-Yaqout and Hamodoa, 2003; Timur and Ozturk (1999); Marttinen et al. (2002); Tatsi et al. (2003)). The composition of the Rainham leachates is similar to that of Hong Kong leachates (JB, GDB) although Rainham is older than the Hong Kong landfill. The low organic contents of the young Hong Kong leachates (JB, GDB) are a result of the high temperature and rainfall which enables a stable methanogenic state to be reached quickly. The Pitsea leachates have higher COD and solids contents than those of Taiwan, Finland and Hong Kong (JB, GDB), similar to those of Kuwait and lower than those of the USA, Germany and Turkey. These differences in leachate composition may be due to the presence of bottom ash in Taiwan landfills, high rainfall and early methanogenic stages developed in Hong Kong landfills and the rising water table in Kuwait landfills. The concentrations of chloride, Na^+ and K^+ of Pitsea and Rainham leachates are significantly higher than the leachates generated in Taiwan and Hong Kong (JB, GDB). The high values of these ions are reflected by the high conductivity in Pitsea and Rainham leachates.

The above results confirm that the chemical composition of leachates may vary significantly from site to site. Differences were found in the concentrations of organic matter, anions and cations and in total and fixed solids contents. Leachates from Pitsea had higher solids, organic matter, sodium, chloride and magnesium contents than leachates from Rainham in all of the untreated and treated samples. In addition, Pitsea and Rainham leachates found to have different characteristics (pH, BOD, COD etc.) in comparison to leachates from other countries. Owing to such a variable characteristics of landfill leachates, it is essential to conduct a long term monitoring programme to obtain representative information on leachates and to understand the detailed evolution of the constituent organic matter.

Table 4.2 Chemical characteristics of leachates from different landfills

Parameter (mg/l)[1]	**Taiwan Site A, B, C** (Fan et al., 2006)	**HK JB, GDB** (Chu et al., 1994)	**Kuwait** (Al-yaqout and Hamodoa, 2003)	**USA** (Al-yaqout and Hamodoa, 2003)	**Germany** (Al-yaqout and Hamodoa, (2003)	**Turkey** (Timur and Ozturk, 1999)	**Finland** (Marttinen et al., 2002)	**Greece** (Tatsi et al., 2003)
Age	10-17	3.5-11	11	-	-	-	-	-
Waste type	Predominantly MSW, some bottom ash	Predominantly MSW, some Hazardous	MSW and construction/demolition waste	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned
pH	7.74-7.91	7.6-7.8	7.55	6	6.9	7.3-7.8	7.1-7.6	7.9
BOD	49.6-173.8		30-600	13400	400-45900	10800-11000	84	1050
COD	690-3038	489-1670	158-9400	1340-18100	1630-63700	16200-20000	340-920	5350
TOC	249-1025							
TS	3941-9620	920-5580						
TVS		498-1580						
TFS		398-4010						
TSS	34-193							480
VSS	51-166							
TDS	3907-9464							
NO_3-N	2.96-26.7	0.06-179						
NH_3-N						1120-2500	330-560	940
Na^+	297-3524	132-1190						
Ca^{2+}	15.9-137.5		5.6-122	254.1-2300	70-290			
Mg^{2+}	15.7-163	9-63	5.2-268	233-410	100-270			

[1] Except pH all parameters are in mg/l

4.3 Effect of a laboratory scale aerobic biological treatment in the change of the nature of recalcitrant organic compounds in landfill leachates

4.3.1 Conventional COD and DOC analyses

The change in the nature of organic compounds in all of the untreated and treated leachate samples during laboratory scale aerobic biological treatment over a period of 30 days is presented in figure 4.1 (a-d). This investigation was carried out using the conventional characterisation methods of COD and DOC.

Figure 4.1 (a, b) shows the total and dissolved COD and total and dissolved organic carbon (TOC, DOC) as a function of time for all of the untreated and treated leachate samples. The results show a gradual decrease of COD and TOC (DOC) with time indicating the reduction of organic compounds by biological processes. However, a slight increase in COD at the beginning of aerobic biodegradation was observed for every leachate sample (figure 4.1 (a)). Similar results have been reported by Nilsum (1998) and Bila et al. (2005) and this might be due to a rapid change in the structure of the organic compounds as a consequence of reactions in the formation of short-term intermediates that are easily oxidizable in the COD test. Figure 4.1 (a, b) also shows that the COD and DOC concentrations were high in the untreated leachates Pitsea (P4) and Rainham (P2), indicating that these two leachates contained significant amounts of organic compounds. The treated leachate Pitsea (LTP) also had high values of COD and DOC. This suggests that the treatment applied prior to aerobic biodegradation in the laboratory and the subsequent aerobic biodegradation were insufficient to remove all of the organic compounds from this particular leachate. These figures and those in table 4.1 also show that the organic compounds in the leachates contributing COD and TOC were mostly in dissolved form.

Figure 4.1 (c, d) shows the percentage removal of COD and DOC in the laboratory scale aerobic biodegradation experiments as a function of time. The %DOC trend shows that after 30 days aeration the degradation of different leachates are in the order of Rainham (LTP) > Rainham (FE) > Pitsea (LTP)> Rainham (LTP Haz) > Pitsea (P4) > Rainham (P2) > thereby indicating their biodegradability. However, from the %DOC results the leachates can be divided into two groups in terms of their biodegradation after 30 days.

The Rainham (LTP) and Rainham (FE) lechates which have low concentrations of organic compounds could be classed as easily biodegradable leachates whereas the rest of the leachates which have high concentrations of organic compounds could be classed as not easily biodegradable. An exception is observed for Rainham (LTP Haz) leachate which exhibits low biodegradability although this leachate has a low amount of organic compounds. This might be due to the presence of chlorinated aliphatic and aromatic organic compounds in this leachate. The %DOC removal found in different leachates was not the exact replica of the observed %COD removal trend. While the highest %DOC removal was observed for Rainham (LTP) leachate the lowest %DOC removal was observed for a different leachate, Rainham (P2). This could be explained by the difference in the method of estimating non-degraded compounds in these two techniques. Due to the presence of the strong oxidizing agent, some inorganic fractions of leachate get incorporated in COD measurements whereas the DOC measurement mostly estimates the organic fraction of leachate. However, from figure 4.1 (c, d) it can be concluded that the biodegradability of Rainham (LTP) leachate is the highest after 30 days of aeration whereas Pitsea (P4) leachate can be accepted as one of the least biodegradable leachates among the six leachates under consideration.

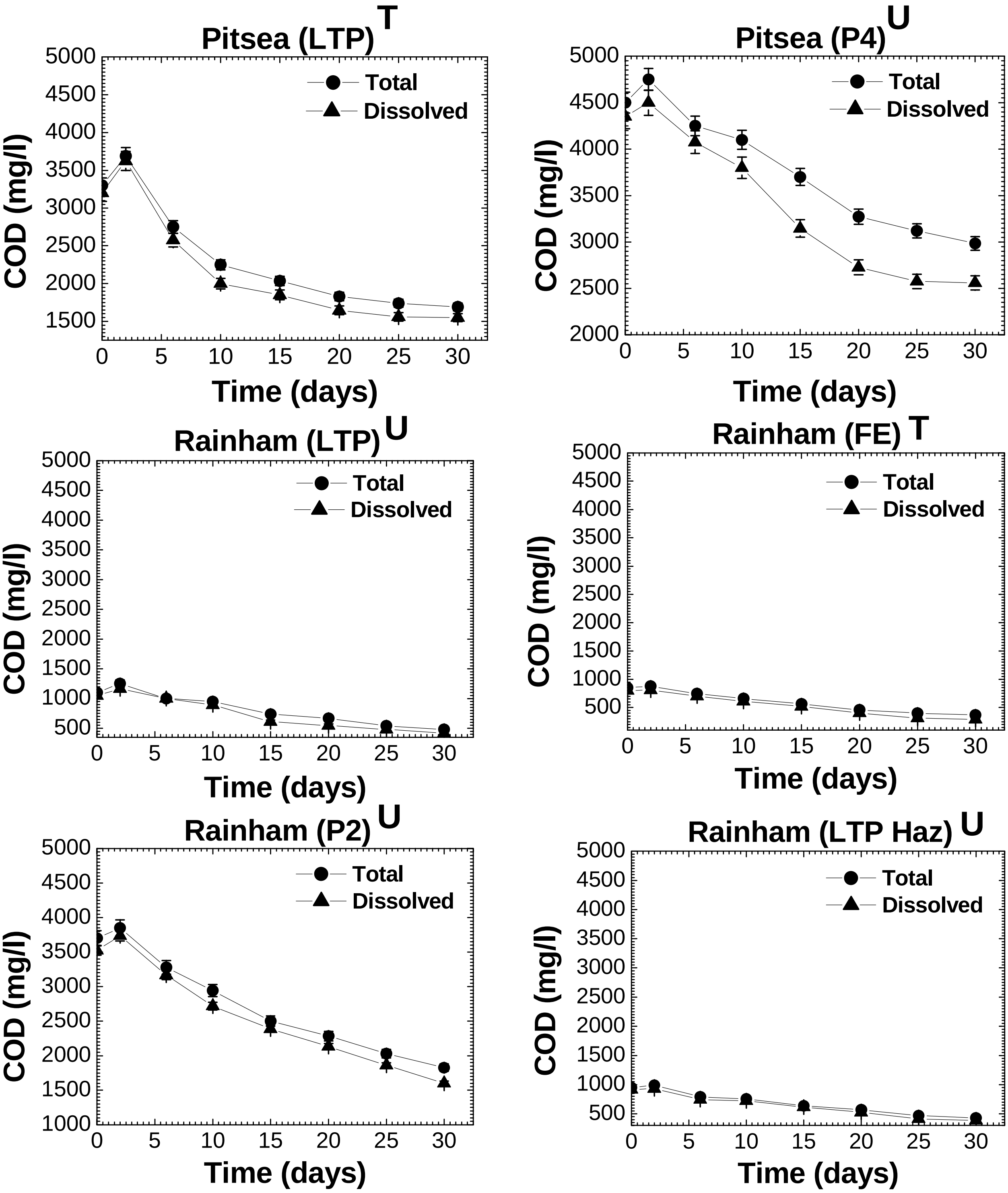


Figure 4.1 (a) The change in COD over time during aerobic biodegradation for all of the untreated and treated leachate samples (T = treated and U = untreated)

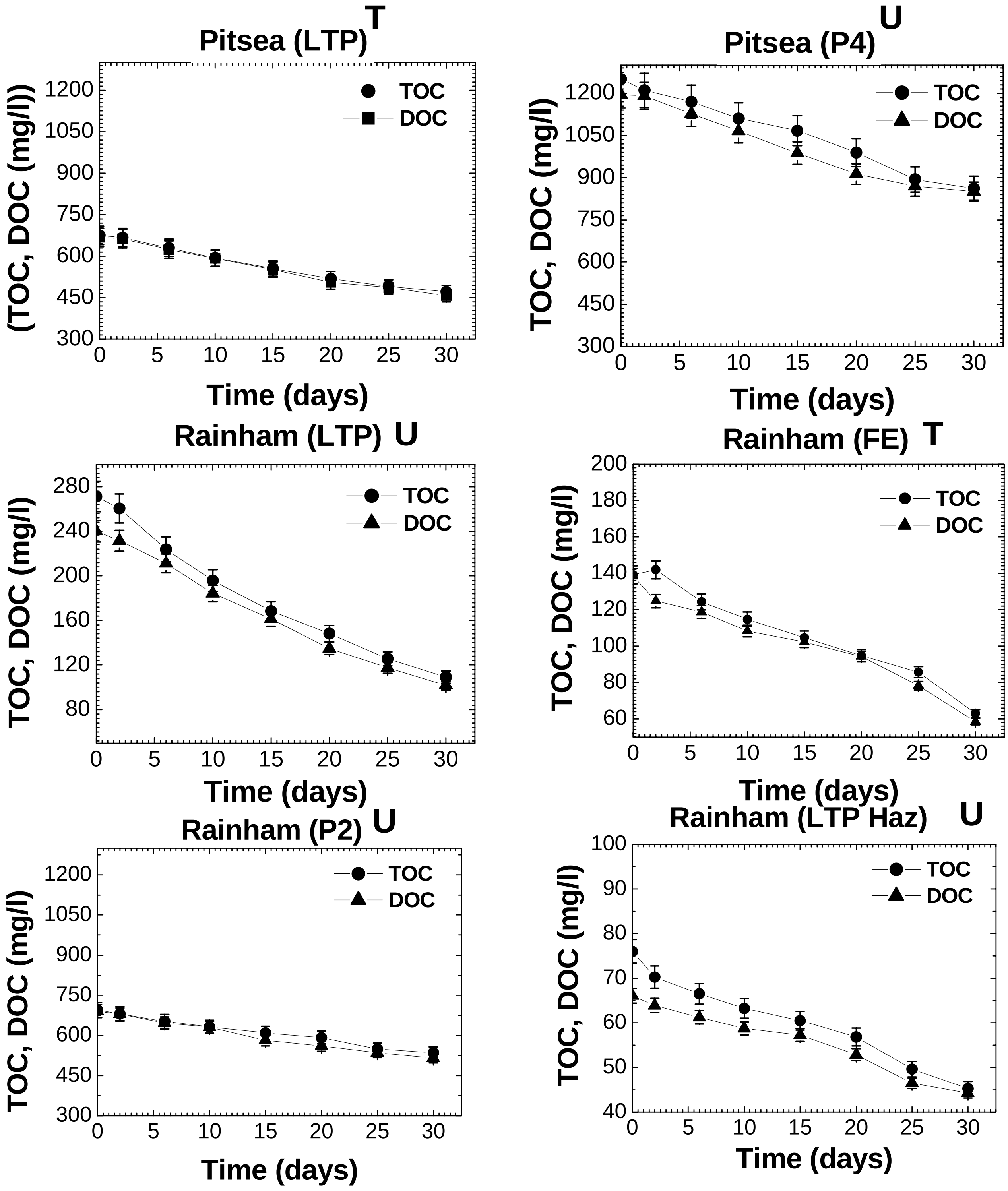


Figure 4.1 (b) The change in TOC and DOC over time during aerobic biodegradation for all of the untreated and treated leachate samples (T = treated and U = untreated)

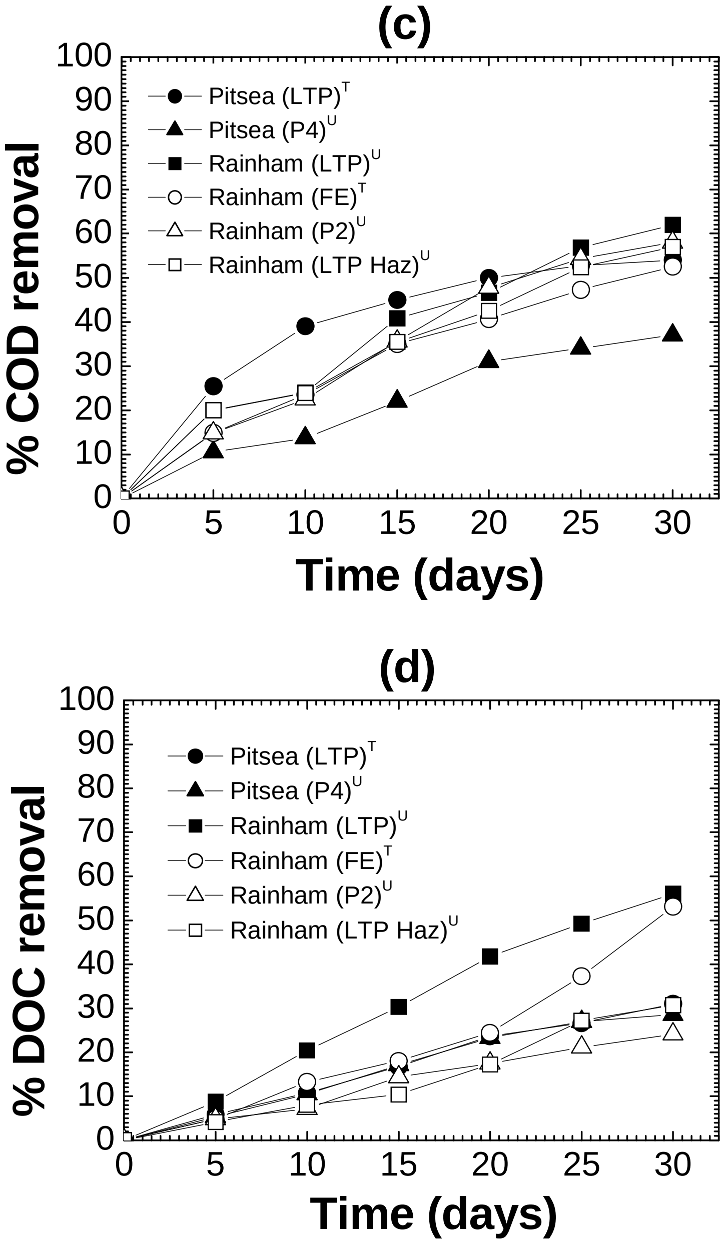


Figure 4.1 Aerobic biodegradation of landfill leachates for 30 days (c) % COD removal; and (d) % DOC removal (T = treated and U = untreated)

The above results also show an increasing amount of removal of degradable compounds with time during aerobic biological treatment of different landfill leachate samples. However, the overall efficiencies and effectiveness of treatment process was found to depend not only on whether the leachates were untreated or treated but also on the variation of leachate composition and landfill site characteristics. For example, the treated leachate Rainham (FE) showed a high percentage removal of COD and DOC in comparison to the untreated leachate Pitsea (P4) (figure 4.1 (c, d)) while it was expected that in general a treated leachate would show a low removal of COD and DOC in comparison to the untreated leachate.

The conventional COD and DOC methods presented in this section were used to estimate the amount of remaining organic compounds in leachates, and the treatment efficiency was evaluated by the percentage removal of the COD and DOC over time. However, these methods were not enough to identify the constituent structure of recalcitrant organic compounds which might have an effect on the treatment of leachates collected from different landfill sites. Therefore, UV and fluorescence spectroscopy were used to characterize the recalcitrant organic compounds by estimating the eventual removal of different organic constituents of leachate in the course of the biodegradation. These investigations study the degradation potential of different organic compounds in leachates and asses the feasibility of using UV and fluorescence spectroscopy for analysing leachates.

4.3.2 Spectroscopic analysis

In this section the nature and characteristics of recalcitrant organic compounds in landfill leachates during aerobic biodegradation were evaluated using UV absorbance and fluorescence spectroscopic techniques. From COD and TOC analyses (figure 4.1 (a, b)) it was found that the organic matter in the leachate samples was mostly in dissolved form. Therefore, unfiltered leachate samples were analysed for their spectroscopic characteristics to obtain the information required.

4.3.2.1 UV spectroscopic results

UV absorption spectroscopy has many useful applications in characterizing the aromatic carbon content. Bari and Farooq (1984), James et al. (1985) and Matsche and Strumworher (1996) reported that UV absorbance at 254 nm wavelength has a close correlation with aromaticity. McKnight et al. (2001) and Abbt-Braun et al. (2004) showed a good correlation between UV_{254} and unsaturated sp^2 hybridized carbon atoms by ^{13C}NMR and suggested UV absorbance at 254 nm wavelength (UV_{254}) as an excellent surrogate parameter for estimating the aromatic organic compounds.

Figure 4.2 (a) shows the change of UV_{254} absorbance over time during aerobic biodegradation for all of the leachate samples. The highest initial value of UV_{254} absorbance was observed in the leachate Pitsea (P4) whereas the lowest value was observed in the Rainham (FE) leachate. Intermediate values of UV_{254} absorbance were observed in Rainham (P2), Pitsea (LTP), Rainham (LTP) and Rainham (LTP Haz) leachates with decreasing order respectively. This suggests that the Pitsea (P4) leachate had the highest amount of aromatic organic compounds, with the other leachates having progressively decreasing amount of aromatic compounds as shown in figure 4.2 (a). It is worth noting that although Rainham (LTP Haz) leachate contained greater concentrations of chlorinated aliphatic and aromatic organic compounds (Table 3.1) than Rainham (LTP) leachate, experimental results showed lower UV_{254} absorbance values for Rainham (LTP Haz) leachate than for Rainham (LTP) leachate. This may be related to the lower DOC value found for the Rainham (LTP Haz) leachate in comparison to the Rainham (LTP) leachate (figure 4.1 (b)). However, possible other reason will be discussed later (section 4.4.2).

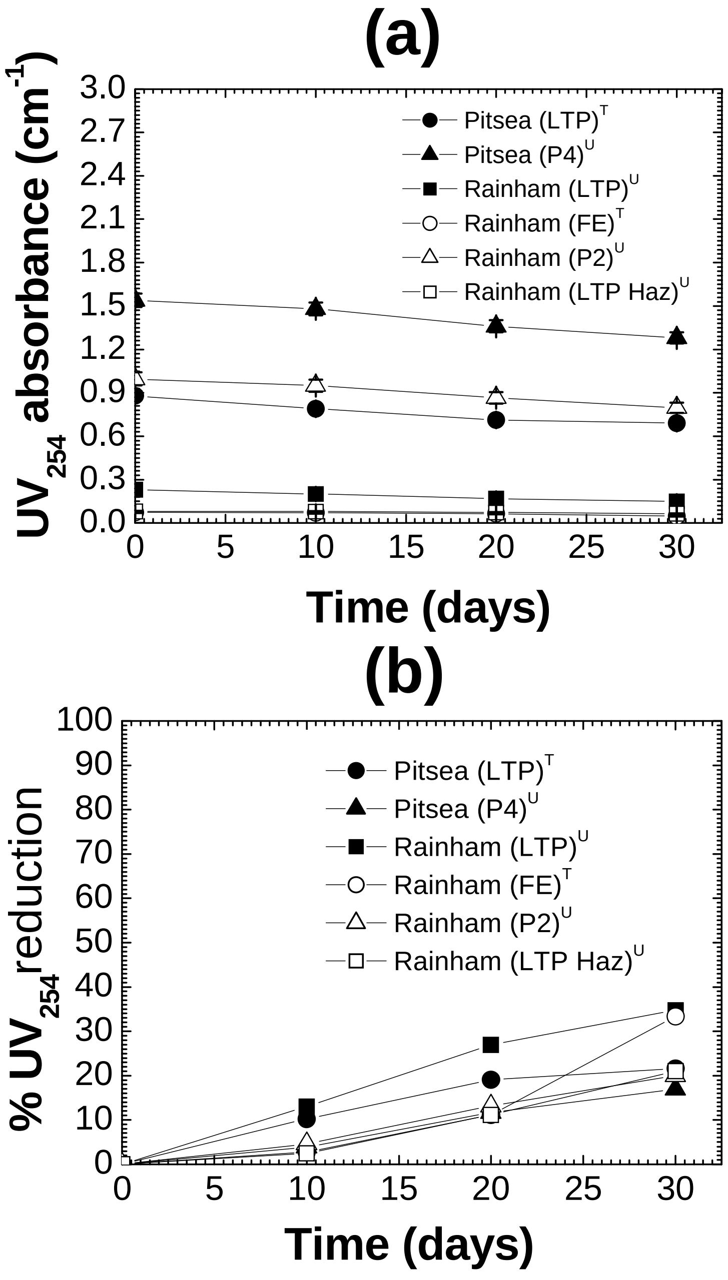


Figure 4.2 (a) Absorbance at 254 nm (UV_{254}) and (b) % UV_{254} reduction for all of the untreated and treated leachate samples (Dilution factor 25) (T = treated and U = untreated)

In all of these leachates, the UV_{254} absorbance decreased with an increasing number of days of aeration. This suggests the breakdown of the aromatic structures of the constituent organic matter by an aerobic treatment process (Gottschalk et al., 2000). However, this might also be attributed to the reaction of oxygen with the unsaturated bonds and aromatic rings, leading to the splitting of bonds in the organic compounds (Gottschalk et al., 2000).

Figure 4.2 (b) shows the percentage reduction of UV_{254} absorbance during aerobic biodegradation for all of the untreated and treated leachate samples. With the exception of Rainham (LTP Haz) leachate, a high percentage of UV_{254} absorbance reduction (figure 4.2 (b)) was observed for leachates with low UV_{254} absorbance values (figure 4.2 (a)). This suggests that leachates containing low aromatic organic compounds had a high percentage reduction of UV_{254} absorbance and vice versa. As discussed before, the deviation for Rainham (LTP Haz) leachate could be explained again by the possible presence of chlorinated aliphatic and aromatic organic compounds. The percentage reduction of UV_{254} absorbance (figure 4.2 (b)) showed the degradation of different leachates in the order of Rainham (LTP) > Rainham (FE) > Pitsea (LTP) Rainham (LTP Haz) > Rainham (P2) > Pitsea (P4) which was in good agreement with the percentage removal of DOC observed in the different leachates (figure 4.1 (d)). Again from the UV_{254} absorbance reduction trend, Rainham (LTP) and Rainham (FE) lechates can be accepted as easily biodegradable leachates whereas the rest of the leachates can accepted as not easily biodegradable. However, the percentage reduction of UV_{254} absorbance in different leachates was not the exact replica of the observed %COD removal trend. This can be explained again by the fact that some inorganic fractions of leachate get incorporated in COD due to the presence of strong oxidizing agent while the UV_{254} absorbance measurable are aromatic organic compounds. Thus, the fairly good agreement on the degradation trend observed in different lecahtes by UV_{254} absorbance and DOC measurements probably indicate that organic compounds contributing DOC in these leachates were mostly aromatic in nature. However, the results of UV spectroscopy and the conventional DOC experiments suggest that leachates containing high aromatic organic compounds usually result in low percentage removal of DOC in the course of a treatment process.

4.3.2.2 Fluorescence spectroscopic results

Figure 4.3 shows the Excitation-Emission-Matrix (EEM) maps of the leachate DOM fractions generated before and after laboratory scale aerobic biodegradation (EEM maps for other days during aeration are presented in Appendix A). Four distinct zones were identified on the EEM maps that were indicative of the presence of different organic substances and are described below. The EEM spectra also show Reyleigh and Raman scattering peaks originating from the interaction of light and water molecules.

Zone 1 (H-L): Peaks were also observed to be present between 230-260 nm and 360-390 nm excitation and 400-460 nm and 460-480 nm emission respectively and is widely recognized as a component of the humic-like fractions (H-L) (Coble, 1996; Baker and Curry, 2004; Cumberland and Baker, 2007), which has also been found in untreated wastewater (Baker et al., 2004) and urban river water (Fu et al., 2007).

Zone 2 (F-L): A peak, which was present in all of the leachates between 320-350 nm excitation and 400-440 nm emission, can be attributed to aromatic and aliphatic groups in the DOM fractions, and is commonly labelled as fulvic-like (F-L) (Coble, 1996; Baker and Curry, 2004). F-L fluorescence has also been detected in lake water (Mostofa and Yoshioka, 2005).

Zone 3 & 4 (Protein-like): Peaks were observed at 230-240 nm and 270-280 nm excitations and 300-320 nm emission, labelled as tyrosine-like (Tyr-L) and at 230-240 nm and 270-280 nm excitations and 340-370 nm emission labelled as tryptophan-like (Trp-L). These peaks are attributed to ‘protein-like’ structures (Coble, 1996; Elliot et al., 2006). ‘Protein-like’ structures have also been widely observed in marine water (Coble, 1996; Ogawa et al., 2001) and river water (Mostofa and Yoshioka, 2005; Fu et al., 2007).

Fluorescence peaks that are ascribed as H-L, F-L and protein-like compounds originate from the degradation of carbohydrate, proteins, fats etc. present in the waste through various physical, chemical and microbiological processes (Calace and Petronio, 1997). It has been suggested that the carbohydrate and protein compounds undergo condensation that leads to the formation of humic-like (H-L) substances according to the melanoidin model (Ikan et al., 1986). Unaltered lignin in the anaerobic environment also contributes towards the formation of recalcitrant H-L and F-L compounds (Komilis and Ham, 2003). As discussed in Chapter 2, microbial synthesis taking place in the landfill can also lead to the formation of in-situ humic and protein-like compounds (Calace and Petronio, 1997; Pichler and Kogel-Knabner, 2000). Previous studies have demonstrated that protein-like compounds (Zone 3 and 4) are associated with bacterial activities as well (Cammack et al., 2004; Elliott et al., 2006). As the H-L and F-L compounds are known as the essential building block of recalcitrant organic compounds (Artiola-Fortuny and Fullar, 1982; Castagnoli et al., 1990 and Christensen et al., 1998), the change of fluorescence peak intensities and peak positioning of recalcitrant H-L and F-L compounds of different untreated and treated leachates from different landfills in the course of treatment may provide important information on the compositional characteristics of the recalcitrant organic compounds of leachates.

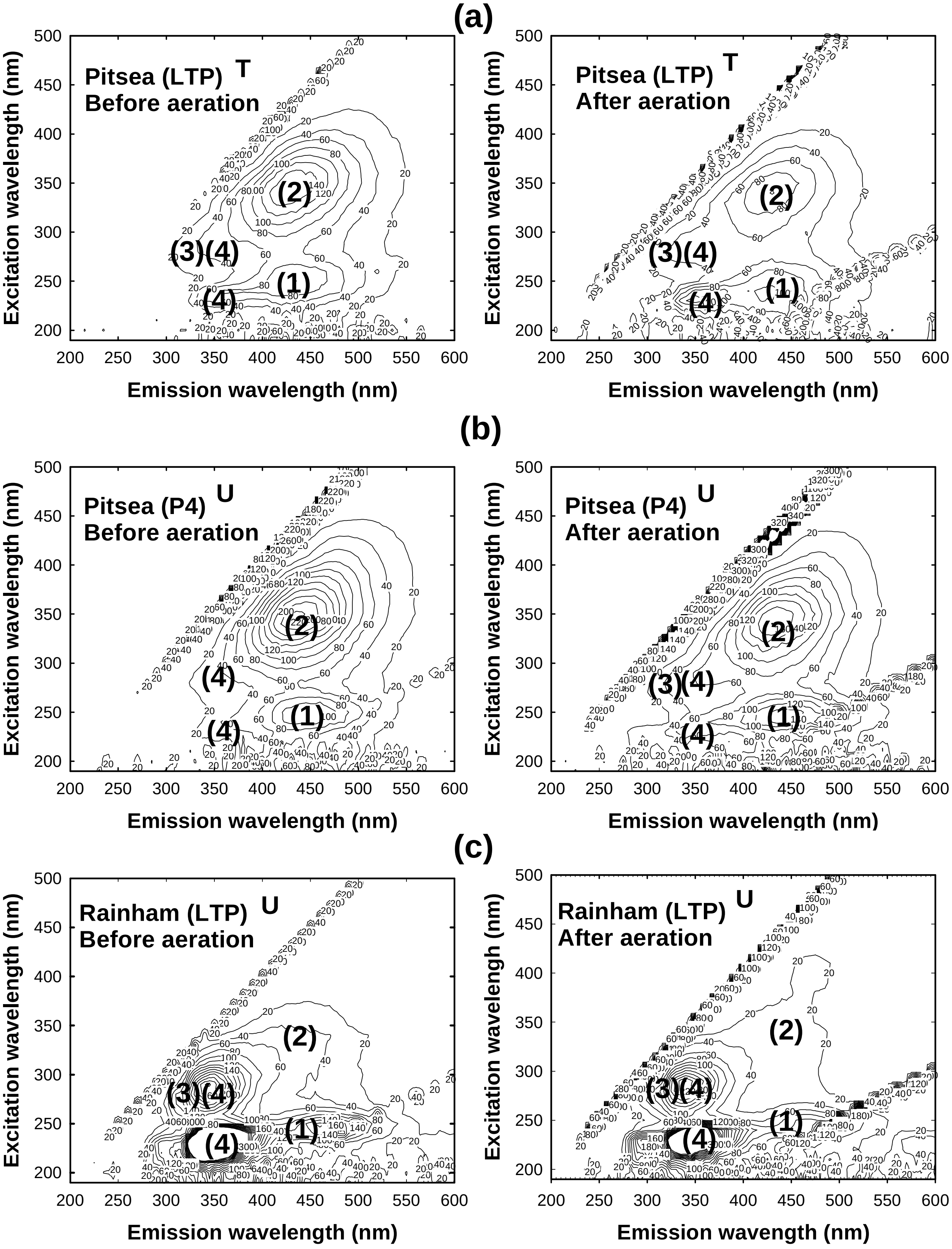


Figure 4.3 Excitation-Emission Matrices (EEM) for landfill leachates before and after aeration (Zone 1 H-L; 2 F-L; 3 and 4 protein like) (dilution factor 25) (T = treated and U = untreated)

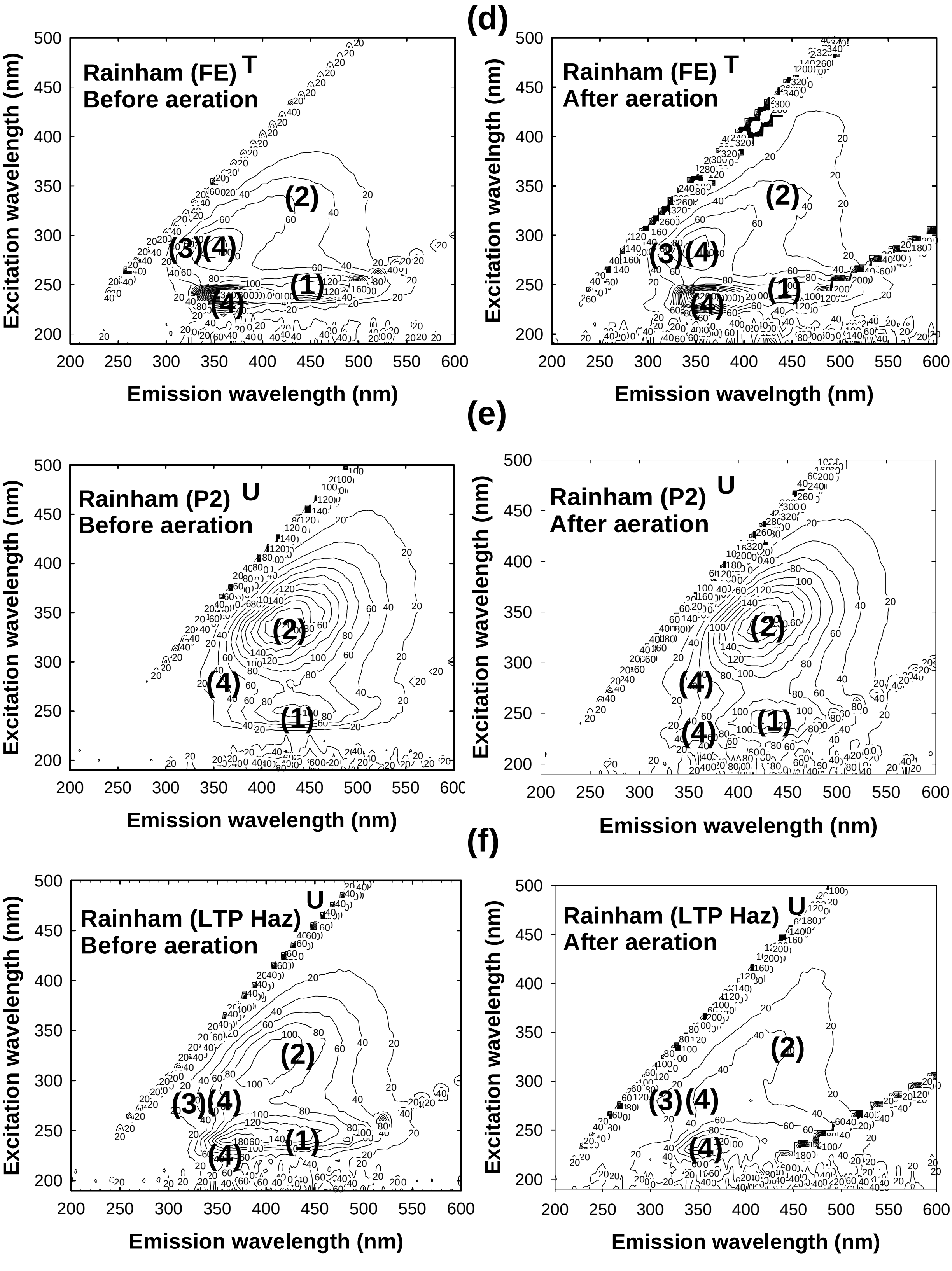


Figure 4.3 (contd.) Excitation-emission matrices (EEM) for landfill leachates before and after aeration (Zone 1 H-L; 2 F-L; 3 and 4 protein like) (dilution factor 25) (T = treated and U = untreated)

Table 4.3 presents the summary of fluorescence properties for all of the untreated and treated leachate samples. Leachates Rainham (LTP), Rainham (FE) and Rainham (LTP Haz) had relatively lower fluorescence intensities in Zones 1 (H-L) and 2 (F-L) and higher intensities in Zone 3 (Tyr-L) and 4 (Trp-L) than leachates Pitsea (LTP), Pitsea (P4) and Rainham (P2) (with the exception of Tyr-L compounds in leachate Rainham (LTP Haz)). This suggests that Rainham (LTP), Rainham (FE) and Rainham (LTP Haz) had relatively lower concentrations of H-L and F-L compounds and higher concentrations of protein-like compounds than leachates Pitsea (LTP), Pitsea (P4) and Rainham (P2). As H-L and F-L compounds are the essential building block of recalcitrant organic compounds, these results indicate that Pitsea (LTP), Pitsea (P4) and Rainham (P2) leachates contained higher concentrations of recalcitrant organic compounds than leachates Rainham (LTP), Rainham (FE) and Rainham (LTP Haz). Table 4.3 and figure 4.3 also show that aerobic biodegradation decreased the intensities of Zone 1 (H-L) and 2 (F-L) materials by about 4-100% in all of the untreated and treated leachate samples over 30 days of period. The decrease in intensities could be explained by the decomposition of H-L and F-L fluorophores with the aerobic biodegradation (Saadi et al., 2006; Uyguner and Bekbolet 2005) thereby indicating the degradation potential of humic and fulvic like compounds of different leachates in Table 4.3.

In Rainham (LTP) and Rainham (FE) leachates a comparatively a high reduction (50-64%) of fluorescence intensities in Zone 1 (H-L) and Zone 2 (F-L) materials was observed. This indicates that aerobic biodegradation had significantly broken down H-L and F-L structures in these two leachates. Rainham (FE) was the final effluent of Rainham (LTP) and Rainham (LTP Haz) (Table 3.1). However, the fluorescence intensities of this leachate in Zone 1 (H-L) and 2 (F-L) before aerobic biodegradation was almost similar to the leachates Rainham (LTP) and Rainham (LTP Haz), indicating that the treatment applied onsite prior to aerobic biodegradation was not effective in removing the recalcitrant organic compounds. In the case of Pitsea (P4) and Rainham (P2) leachates, aeration reduced the intensities of Zone 1 (H-L) and 2 (F-L) materials by only about 4-13%, indicating that a considerable fraction of high molecular weight organic compounds remained. Although Rainham (LTP Haz) leachate is to be enriched with chlorinated aliphatic and aromatic organic compounds (Table 3.1), aeration reduced the intensity of Zone 1 (H-L) materials by 100% after 10 days. This might be attributed to the selfquenching within H-L molecules (Chen et al., 2003) or by internal quenching by other organic or inorganic molecule intermediates formed during the biodegradation/remineralisation process (Saadi et al., 2006). Senesi (1990) also attributed this effect to the greater proximity of aromatic choromophores and the consequent greater probability of deactivation of excited states by internal quenching in higher molecular weight molecules. This also may explain the low UV_{254} absorbance observed in this leachate (section 4.4.1).

Table 4.3 Fluorescence properties of landfill leachate samples during aerobic treatment (Total fluorescence intensity was calculated by the cumulative integration of the intensity versus emission wavelength data for any given excitation wavelength range corresponding to different types of materials and was corrected by multiplying by dilution factor 25) (SD = standard deviation, n=3)

Leachate sample	No. of days after aeration	Zone 1	Zone 2	Zone 3	Zone 4	
		Humic like fluorescence at 230-260 nm and 360-390 nm excitation and 400-460 nm and 460-480 nm emission respectively	**Fulvic like fluorescence at 320-350 nm excitation and 400-440 nm emission**	**Tyrosine at 230-240 nm and 270-280 nm excitation and 300-320 nm emission**	**Tryptophan at 270-280 nm excitation and 340-370 nm emission**	**Tryptophan at 230-240 nm excitation and 340-370 nm emission**
		FI. Intensity (Mean/SD)	**FI. Intensity (Mean/SD)**	**FI. Intensity (Mean/SD)**	**FI. Intensity (Mean/SD)**	**FI. Intensity (Mean/SD)**
Pitsea (LTP) (Treated)	0	1472200/6.5	907675/7.6	10475/4.3	96575/5.5	122750/5.6
	10	1279875/6.3	633775/6.7	10350/4.2	94600/6.5	217275/6.6
	20	1247425/5.5	602375/7.0	10300/5.4	93975/6.8	224300/6.0
	30	1181350/4.6	563475/5.8	7575/5.0	38825/6.9	217950/5.5
% reduction		20	38	28	60	
Pitsea (P4) (Untreated)	0	1967750/2.2	1045125/2.3		154875/3.8	148725/6.6
	10	1843825/2.4	1000000/5.3	14475/3.4	129025/3.7	147750/6.5
	20	1797275/3.5	994600/5.4	13650/4.4	118125/4.4	147400/4.4
	30	1718075/3.3	960400/2.3	11900/4.5	104000/7.5	137900/5.8
% reduction		13	8	18	33	
Rainham (LTP) (Untreated)	0	1399925/7.6	494875/6.8	31925/5.7	3658750/5.7	1566900/6.6
	10	824375/9.0	241550/10.0	31800/9.4	3085000/6.5	11727508.7
	20	748475/9.5	215775/10.0	29175/3.7	2821750/9.0	1164700/8.9
	30	701500/5.2	195750/6.9	27875/7.6	1129250/6.5	1089275/9.0
% reduction		50	60	13	69	
Rainham (FE) (Treated)	0	1347950/4.8	626925/6.9	18375/5.6	1755500/5.5	373425/6.6
	10	795850/9.7	337350/8.4	17300/6.6	1753750/9.8	457000/7.6
	20	712500/5.7	288050/7.6	14950/7.4	1502000/10.0	428000/4.9
	30	562775/7.8	225900/8.4	12400/7.8	505500/10.6	357050/6.0
% reduction		58	64	33	71	
Rainham (P2) (Untreated)	0	1943600/2.3	1067300/3.1		105125/10.7	
	10	1868150/5.5	1047850/2.2		97075/9.4	15475/6.6
	20	1821725/4.3	1028600/2.4		96200/4.8	14550/4.6
	30	1779775/3.9	1025850/3.2		76800/5.9	12300/6.8
% reduction		8	4		27	
Rainham (LTP HAZ) (Untreated)	0	1388100/4.8	645600/7.5	8100/4.6	1554750/7.5	217975/8.7
	10		379300/2.5	6850/5.5	1024250/5.9	260625/7.8
	20		304525/4.5	6650/4.6	912500/6.9	241600/8.8
	30		259975/5.5	5750/5.6	500000/6.8	240450/5.9
% reduction		100	60	29	68	

Fluorescence results also show that the intensities of Zone 3 and 4 (270-280 nm excitation) fluorophores (tyrosine-like and tryptophan-like compounds respectively) decreased during 30 days of aerobic biodegradation. This reduction can be attributed to the breakdown of proteineous materials by aeration (Zhang et al., 2008). However, an increase in fluorescence intensity was observed for Zone 4 (tryptophan-like compounds at 230-240 nm excitation), particularly over the first 10 days for the leachates Pitsea (LTP), Rainham (FE) and Rainham (LTP Haz) (Table 4.3). This trend may be attributed to the enrichment of protein-like compounds during DOC biodegradation. It has been reported that proteins may be preserved by encapsulation into recalcitrant humic molecules (Dinel et al., 1996). Therefore, it seems likely that the humic bound proteineous compounds might have been exposed during aerobic biodegradation thereby increasing the fluorescence intensity in this zone. Table 4.3 also shows that the percentage removal of Trp-L compounds was higher than for the H-L and F-L compounds in all leachate samples (except for Rainham (LTP Haz) leachate). It is interesting to note that before biodegradation (at day 0) the intensities of Tyr-L and Trp-L compounds for leachate Rainham (FE) was significantly lower than leachate Rainham (LTP). As Rainham (FE) was the final effluent in the treatment of Rainham (LTP) and Rainham (LTP Haz), it can be said that the applied treatment onsite was more effective in removing protein-like compounds than H-L and F-L compounds. This indicates that protein-like compounds were more biodegradable than H-L and F-L compounds and therefore aerobic biodegradation was more effective in removing Trp-L compounds than the H-L and F-L compounds. Similar results have been reported by Ahmad and Reynolds (1995, 1999), Reynolds and Ahmad (1997), and Reynolds (2002). Their results suggested that Trp-L compounds are the more biodegradable fractions of DOM and they showed up to 90% reduction in fluorescence intensity of Trp-L compounds from influent to effluent across a treatment process.

Despite the intensity reduction of the H-L and F-L materials, aeration also caused peak-shift for DOM fractions of leachates. In particular, aeration shifted the excitation wavelengths of Zone 2 (F-L) materials of Rainham (LTP) (figure 4.3 (c)), Rainham (FE) (figure 4.3 (d)) and Rainham (LTP Haz) (figure 4.3 (f)) leachates to longer excitation wavelengths. This may be attributed to the enhanced oxidation of the Zone 2 (F-L) materials of these three leachates producing more carbonyl, carboxyl, hydroxyl and amino groups in the structures of the materials thereby indicting their biodegradability (Uyguner and Bekbolet, 2005; Zhang et al., 2008). In the case of Pitsea (P4) (figure 4.3 (b)) and

Rainham (P2) (figure 4.3 (e)) leachates, aeration slightly expanded the EEM peaks of Zone 1 (H-L) and 2 (F-L) materials to shorter emission wavelengths. These shifts may be attributed to the following aeration induced changes for the Zone 1 (H-L) and 2 (F-L) materials (Coble, 1996; Uyguner and Bekbolet, 2005; Zhang et al., 2008):

(1) the decomposition of condensed aromatic molecules to simpler and smaller aromatic or aliphatic molecules;

(2) the reduction in the degree of π-electron systems resulting for example, from a decrease the number of aromatic rings or conjugated bonds in chain structures;

(3) the elimination of certain functional groups such as carbonyl, carboxyl, hydroxyl and amine.

The above results demonstrate that the fluorescence spectroscopy can be used to detect the presence of different organic compounds in leachates. The relatively low reduction of florescence intensities (Table 4.3) in H-L and F-L compounds during aerobic biodegradation simply imply that these materials were the key components of recalcitrant organic compounds whereas high intensity reduction in protein-like compounds suggest that protein-like compounds were more biodegradable. Florescence spectroscopic results also show that leachates from Pitsea exhibited lower intensity reduction of H-L and F-L compounds in comparison to the Rainham leachates (except for Rainham (P2)), indicating that Pitsea leachates may in general be less biodegradable than Rainham leachates. This agrees with the results of conventional COD, DOC, UV_{254} absorbance analysis. In addition to studying the biodegradation of different organic compounds in leachates, florescence spectroscopy can also be used to investigate the structure of the H-L and F-L compounds (recalcitrant compounds) that might affect biodegradation significantly. This is addressed in the following section (Section 4.4.3).

4.3.2.3 Fluorescence analyses of humic and fulvic-like compounds in emission scanning mode

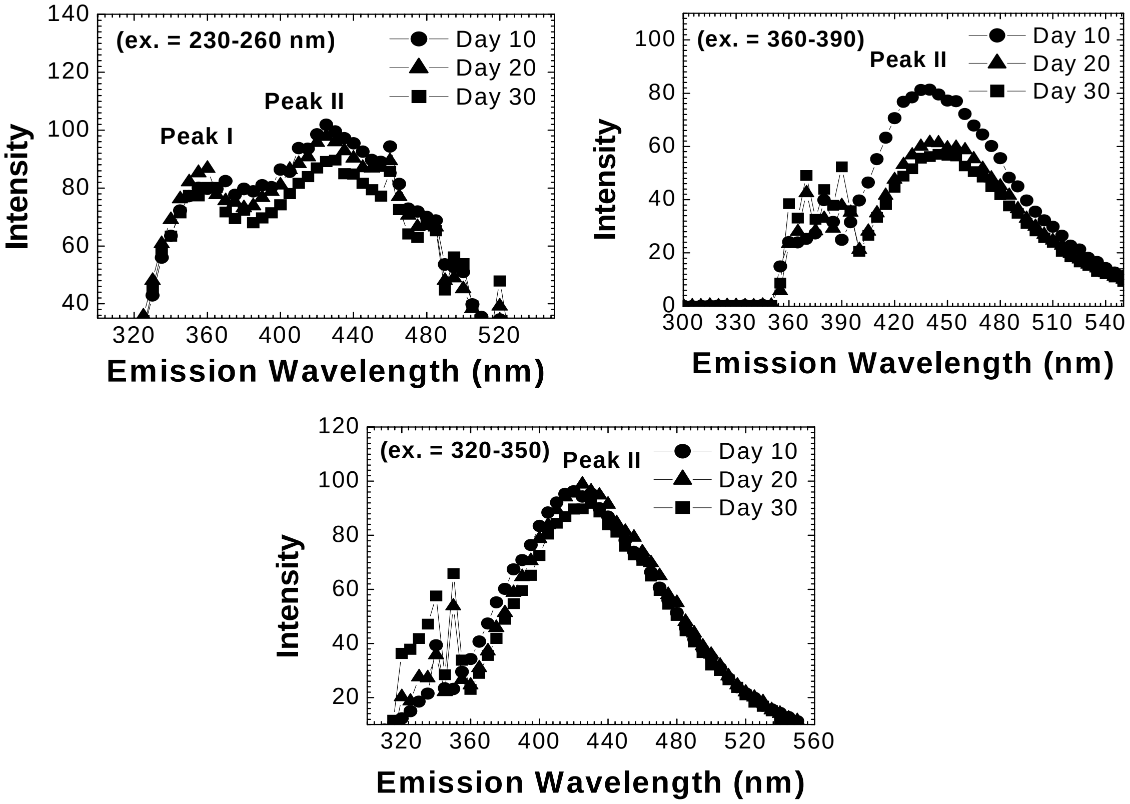
In this section the structure of fluorescent compounds in leachates during aerobic biological treatment is discussed. Fluorescence spectra in emission scanning modes provide information about the aromatic structures with peak positions. Figure 4.4 presents the fluorescence emission characteristics at specified excitation wavelengths of all of the untreated and treated leachate samples. All of the leachates exhibited mainly two peaks, labelled as Peak I at 360 nm and Peak II at 430 nm wavelengths. For all Pitsea leachates, Peak II at (320-350) nm excitation was the strongest whereas for leachates Rainham (LTP) and Rainham (FE), Peak I at (230-260) nm excitation was the strongest. Peak II at (360-390) nm excitation for leachates Pitsea (LTP), Pitsea (P4) and Rainham (P2) also had high intensities. As compared with the EEM spectrum (figure 4.3), Peak I at (230-260) nm excitation can be assigned as tryptophan-like fluorophores. Peak II at (230-260) nm and (360-390) nm excitations can be assigned as H-L and at (320-350) nm excitations as F-L fluorophores. For leachates, Rainham (LTP) and Rainham (FE), Peak III with low intensity is also observed at wavelength of (450-470) nm which can not be found in the EEM spectra (figure 4.3). This Peak III is probably attributed to ‘red shifted’ high molecular weight H-L fluorophores (Chen et al., 2003).

Kang et al. (2002) reported that the shorter wavelength peaks (360 nm) can be interpreted to be due mainly to the presence of simple aromatic ring in the molecule, whereas the peaks in the longer wavelength (430 nm) are due to the condensed aromatic rings and conjugation of simple aromatic rings. It can be seen from figure 4.4 that leachates Pitsea (LTP), Pitsea (P4) and Rainham (P2) showed strong intensities at 430 nm (Peak II), suggesting that these leachates were associated with the presence of linearly condensed aromatic rings of H-L and F-L molecules. However, it is also observed that leachates Rainham (LTP) and Rainham (FE) showed the highest peaks at 360 nm (Peak I) (figure 4.4). This can be ascribed to the presence of simple aromatic rings. Figure 4.4 also shows that aeration reduced the intensities of Peak I and Peak II in all of the leachates. This decrease can be attributed to the destruction of aromatic ring structures through aerobic biodegradation. However, aeration also increases the intensities of Peak I for Pitsea (LTP) leachate. This can be ascribed to the enrichment of the simpler aromatic tryptophan-like structures of Pitsea (LTP) leachate during aerobic biodegradation. This result is also consistent with the EEM spectra (figure 4.3 and Table 4.4).

It was also found that the ratio of intensities of longer to shorter wavelengths for leachate samples Pitsea (LTP), Pitsea (P4) and Rainham (P2) were higher than those of leachate samples Rainham (LTP) and Rainham (FE). This implies that leachate samples Pitsea (LTP), Pitsea (P4) and Rainham (P2) contained aromatic ring of more condensed form than those for leachate samples Rainham (LTP) and Rainham (FE). This may explain the relatively higher intensity reduction for peak I and II of Rainham (LTP) and Rainham (FE) leachates in comparison with Pitsea (LTP), Pitsea (P4) and Rainham (P2) leachates.

The fluorescence spectroscopic results can be correlated with the general trend observed in the UV spectroscopic results and also with the percentage reduction of COD and DOC. Spectral analyses (figure 4.4) indicated that Rainham (LTP) and Rainham (FE) leachates may have contained simple aromatic rings in the molecules. The amount of aromatic organic compounds was also indicated to be low in these leachates (figure 4.2 (a)). These leachates eventually exhibited high percentage reduction of COD, DOC (figure 4.1 (c, d)), UV_{254} absorbance (figure 4.2 (b)) and H-L and F-L intensities (Table 4.4) during aerobic treatment. This allows us to conclude that leachates containing low concentration of aromatic organic compounds and simple aromatic structures may be easily degradable.

Pitsea (LTP) ^{T}



Pitsea (P4) ^{U}

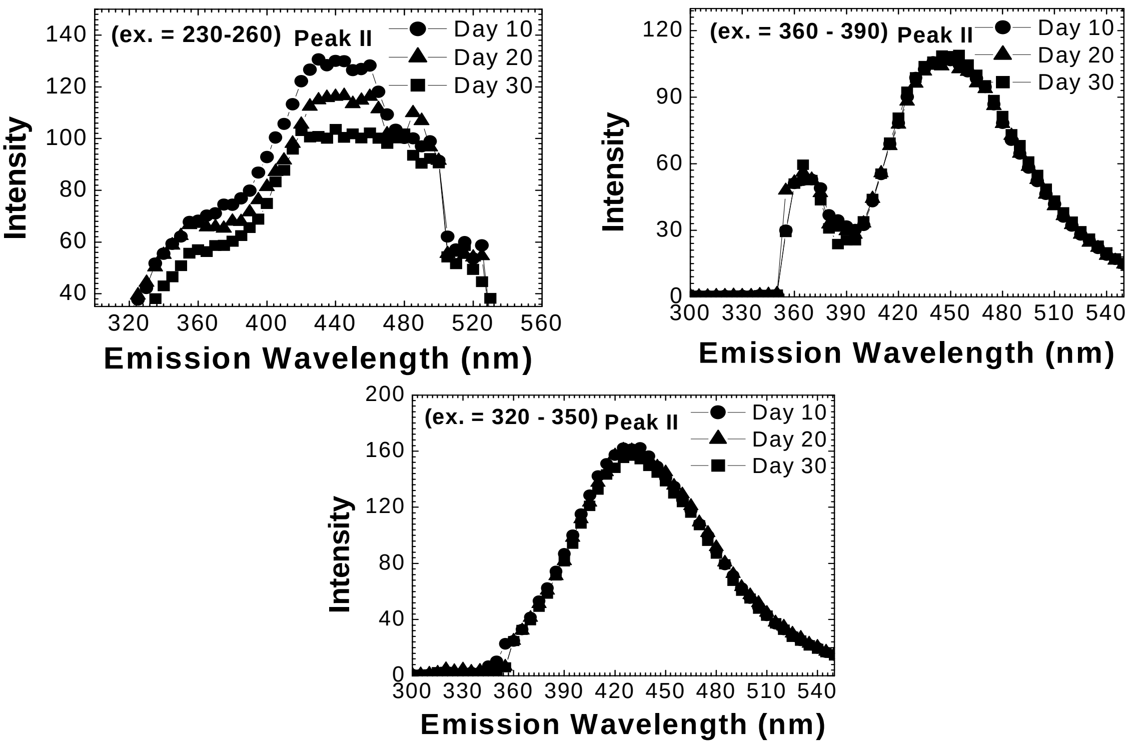
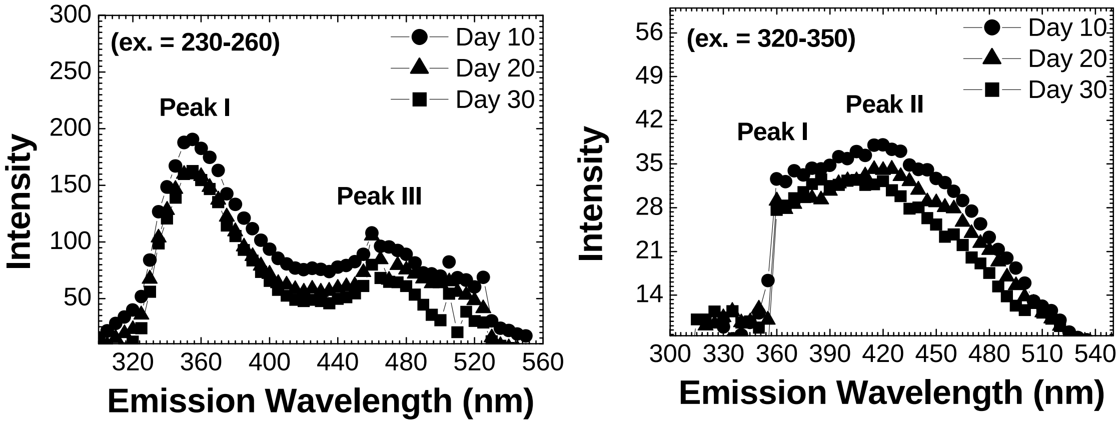


Figure 4.4 Fluorescence spectra in emission scanning mode for all of the untreated and treated leachate samples (dilution factor 25) (T = treated and U = untreated)

Rainham (LTP) U



Rainham (FE) T

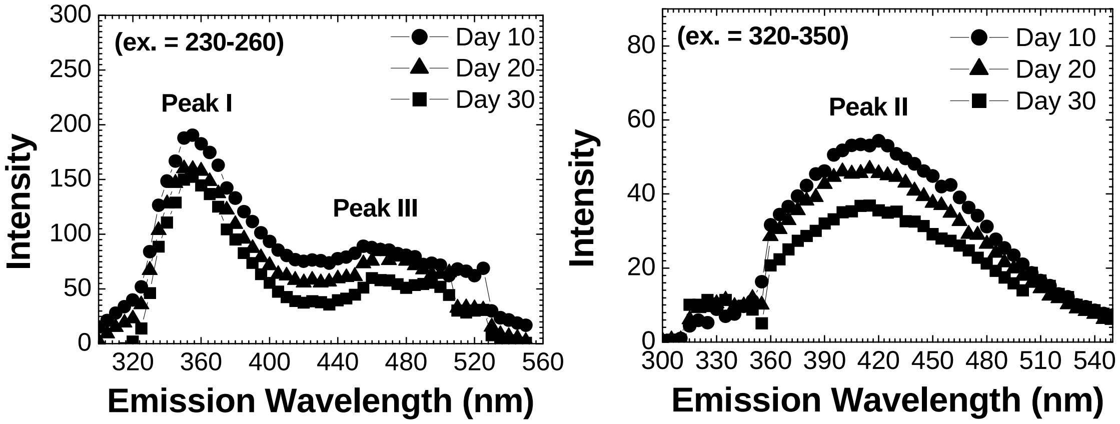
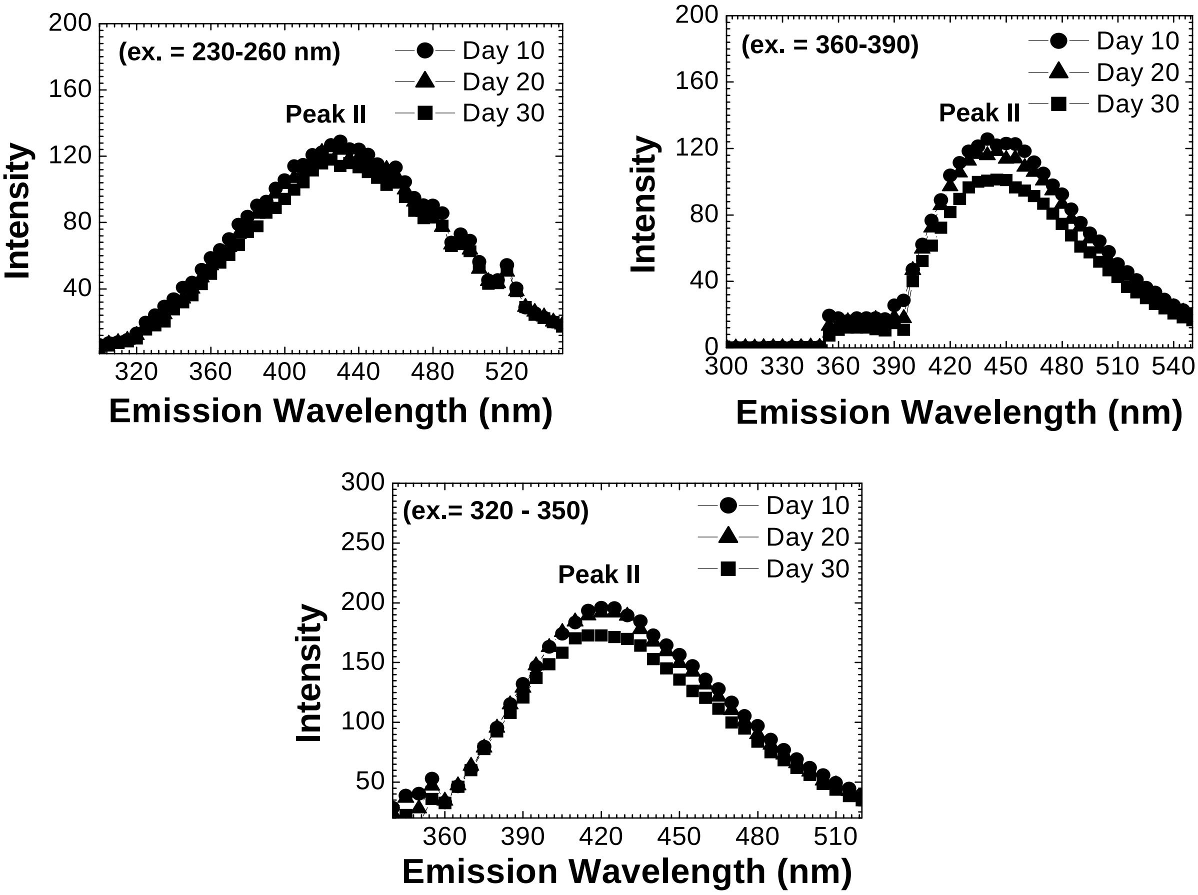


Figure 4.4 (contd.) Fluorescence spectra in emission scanning mode all of the untreated and treated leachate samples (dilution factor 25) (T = treated and U = untreated)

Rainham (P2)^{U}



Rainham (LTP Haz)^{U}

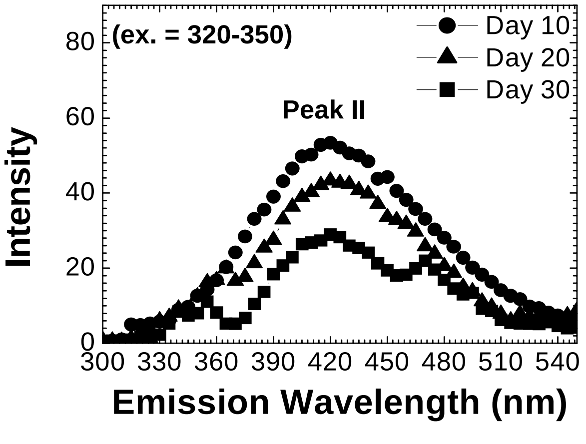


Figure 4.4 (contd.) Fluorescence spectra in emission scanning mode all of the untreated and treated leachate samples (dilution factor 25) (T = treated and U = untreated)

4.4 Feasibility assessment of spectroscopic method

A feasibility assessment of the rapid characterisation of leachates using UV and fluorescence spectroscopy can be made if the biodegradation potential of different leachates obtained by spectroscopic method is compared with the results of well established COD and DOC methods. The biodegradability of different leachates found from %UV_{254} and %DOC reduction experiments were in good agreement with each other. Both these methods showed that Rainham (LTP) and Rainham (FE) lechates were easily biodegradable whereas the rest of the leachates were not so easily biodegradable. The biodegradation study of constituent organic compounds using fluorescence spectroscopy in different leachates was also in agreement with %DOC removal. Fluorescence spectroscopy also indicated that after 30 days of aeration Rainham (LTP) and Rainham (FE) had high degradability whereas Pitsea (LTP), Pitsea (P4) and Rainham (P2) showed relatively low degradability.

Although %COD removal results were not an exact replica of %DOC removal and spectroscopic results, in general this can also be accepted as fair agreement, as %COD removal experiment also showed a high degradability for Rainham (LTP) leachate and low degradability for Pitsea (P4) leachate. Mild inconsistency between established COD and novel methods on a case-by-case basis can be attributed to the difference in the method of estimating non-degraded compounds. As discussed before, due to the presence of a strong oxidizing agent, some inorganic fractions of leachate get incorporated in COD measurements whereas the DOC measurement mostly estimates the organic fraction of leachate. The spectroscopic methods were also organic in nature whereby UV absorption spectroscopy gave a quick estimation on the degradation of aromatic compounds and fluorescence spectroscopy indicated degradation of several different organic compounds.

As a result, the good agreement between spectroscopic method and well established DOC method point to the fact that a rapid assessment of biodegradation potential of leachates using spectroscopic method was in general reliable. In addition, the UV spectroscopy allowed the study of the possible biodegradation of aromatic compounds whereas fluorescence spectroscopy enabled the study of the proportion of humic and fulvic-like materials and their degradation in leachates. As such, these novel methods provided an insight into the recalcitrant organic compounds in these landfill leachates.

4.5 Relationship of spectroscopic methods with COD and DOC

The relationship between fluorescence intensity, UV absorbance at 254 nm wavelength and chemical and biological leachate characterisation parameters (COD and DOC) are established in the following section for individual leachates.

4.5.1 Relationship between fluorescence intensity and DOC

The H- L and F- L fluorescence intensities are plotted against DOC and are presented in figure 4.5 for all of the untreated and treated leachate samples. Linear correlation coefficients are also presented. The results show that relationship between fluorescence intensity and DOC varied among leachate samples. Leachate Rainham (P2) gave the best relation with R^2 = 0.97 and 0.94 for H- L and F- L fluorescence respectively, with the Rainham (FE) leachate giving the weakest relation (H- L, R^2 = 0.72 and F- L, R^2 = 0.7). Intermediate values were found for Pitsea (P4), Pitsea (LTP), Rainham (LTP) and Rainham (LTP Haz) leachates (figure 4.5).

The strongest relationship observed in the Rainham (P2) leachate (figure 4.5) can be attributed to the presence of significant proportions of H- L and F- L materials, which dominate the DOC pool. In contrast, the weakest relationship occurred in the Rainham (FE) leachate, where, H- L and F- L materials probably made up a relatively small component of the DOC (Table 4.4). The weak relationship observed in this leachate could be attributed to the relative insignificance of the H-L and F-L fractions as a proportions of DOC compared with other fluorophores such as Trp-L fractions. Baker (2002) investigated a small urban catchment where the relationship between F-L and DOC for the whole catchment was 0.68, with a stronger relationship between these two parameters in a tributary containing greater proportions of natural DOM but a weaker association between F-L and DOC in the subcatchments where the anthropogenic influences were strong. Similarly Cumberland and Baker (2007) determined the F-L to DOC correlation in river, wetland, spring, pond and sewage samples with strong relationship (R^2 = 0.756) between F-L and DOC in the wetland water samples containing greater proportions of natural DOM but a poor relationship (R^2 = 0.14) between the two parameters in the final treated sewage effluent. The results of this study in a sense contradict the results of Baker (2002) and Cumberland and Baker (2007), where the relationship between H-L/F-L substances and DOC were observed to be stronger for the anthropogenic influences. This might be due to the fact that municipal waste might play a significant role as a source for H-L and F-L compounds.

It is well known that Trp-L fluorescence intensity can be considered as a relic of anthropogenic material in natural water, leachates and even in treated effluents (Galapate et al., 1998; Baker et al., 2003, 2004; Reynolds, 2003). The relationship between Trp-L fluorescence intensity and DOC is presented in figure 4.6 for all of the untreated and treated leachate samples. When considering the relationship between the Trp-L fluorescence intensity and H-L/F-L fluorescence intensity (figure 4.7) significant relationship were also observed. This suggests that both H-L and F-L compounds and protein-like (Trp-L) compounds played a significant role in the DOC of landfill leachates. Furthermore, this indicates that the municipal waste might act as the origin not only of the Trp-L fluorescence substance but also of the H-L and F-L fluorescence substance. That is why in this study, the H-L/F-L fluorescence- DOC relationship was found to be stronger for the anthropogenic leachates.

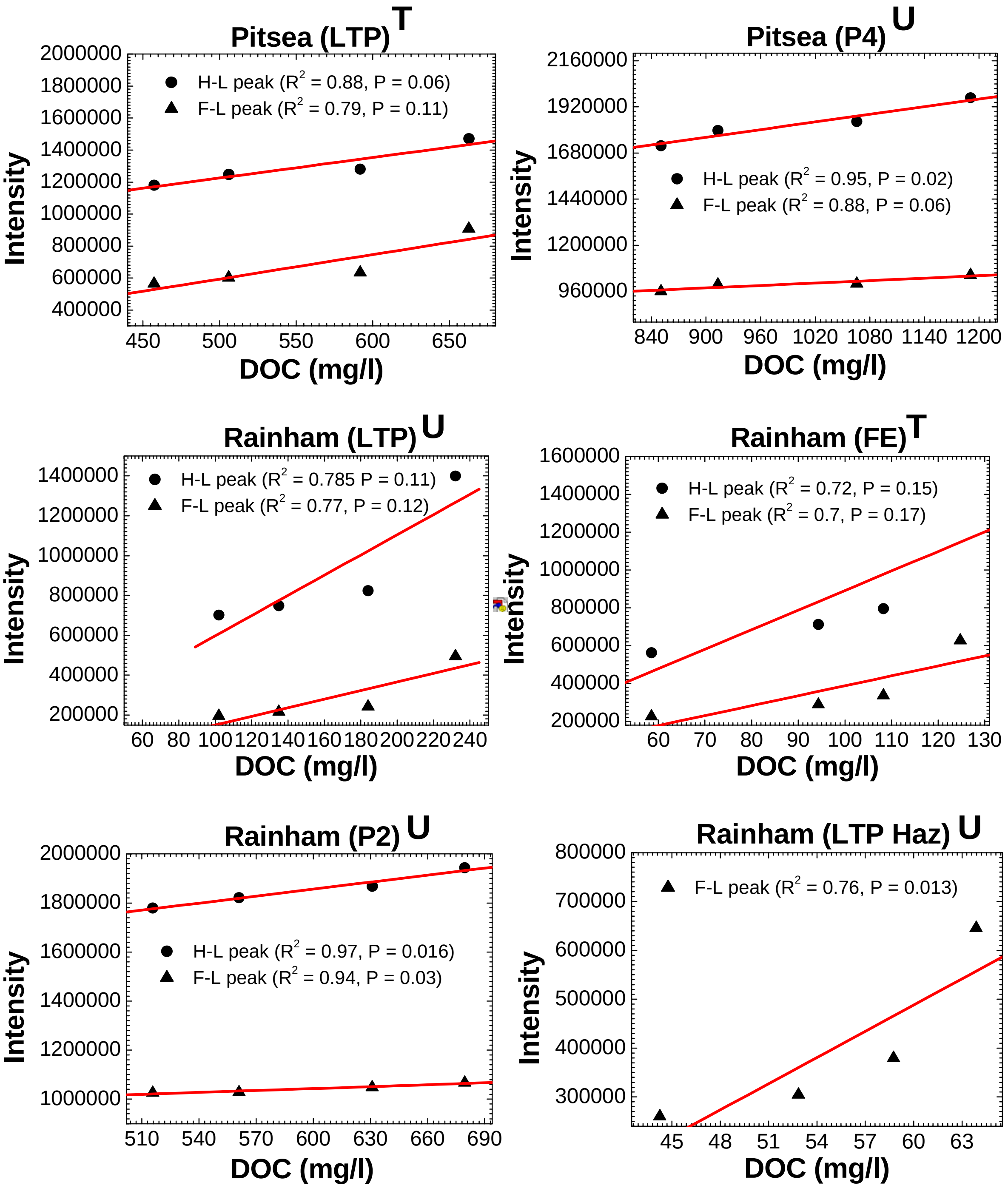


Figure 4.5 Comparisons of the fluorescence peak with DOC for: Pitsea (LTP), H-L: y = 583187 + 1284x, F-L: y = 312561 + 558x; Pitsea (P4), H-L: y = 1.2e6 + 663x, F-L: y = 786330 + 213x; Rainham (LTP), H-L: y = 90453 + 5073x, F-L: y = 142004 + 833x; Rainham (FE), H-L: y = 449708 + 4520x, F-L: y = 244141 + 2454x; Rainham (P2), H-L: y = 1.3e6 + 950x, F-L: y = 888865 + 257x; Rainham (LTP Haz), F-L: y = 391124 + 7058x. (P = probability that R = 0) (T = treated and U = untreated) (Note: non-zero axes are intercepts)

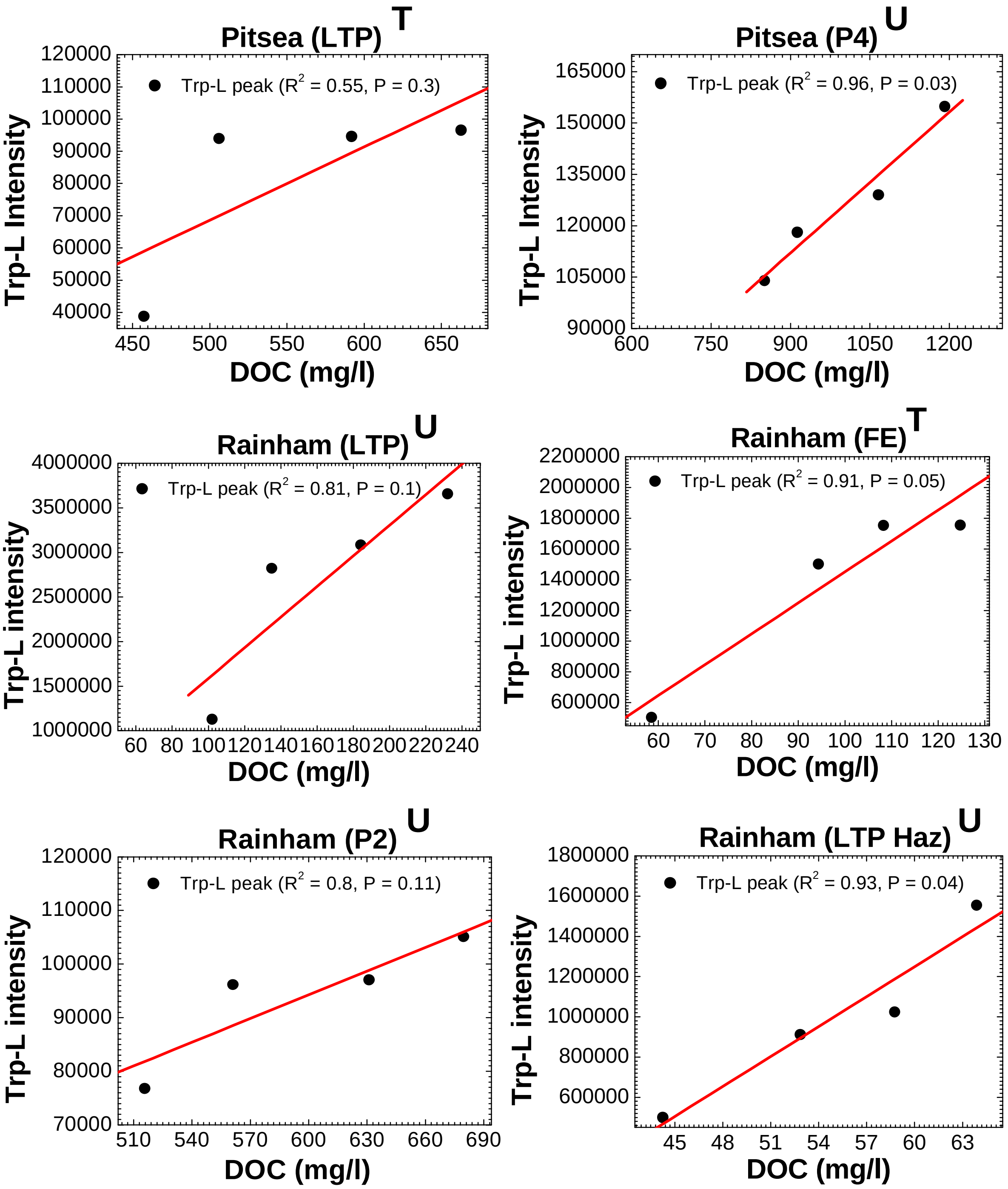


Figure 4.6 Comparisons of the Trp-L (270-280 nm excitation) fluorescence with DOC for; Pitsea (LTP), y = 82788 + 148x; Pitsea (P4), y = -11344 + 137x; Rainham (LTP), y = -129431 + 17171x; Rainham (FE), y = -560439 + 20110x; Rainham (P2), y = 5753 + 148x; Rainham (LTP Haz) y = -1.73e6 + 49658x. (P = probability that R = 0) (T = treated and U = untreated) (Note: non-zero axes are intercepts)

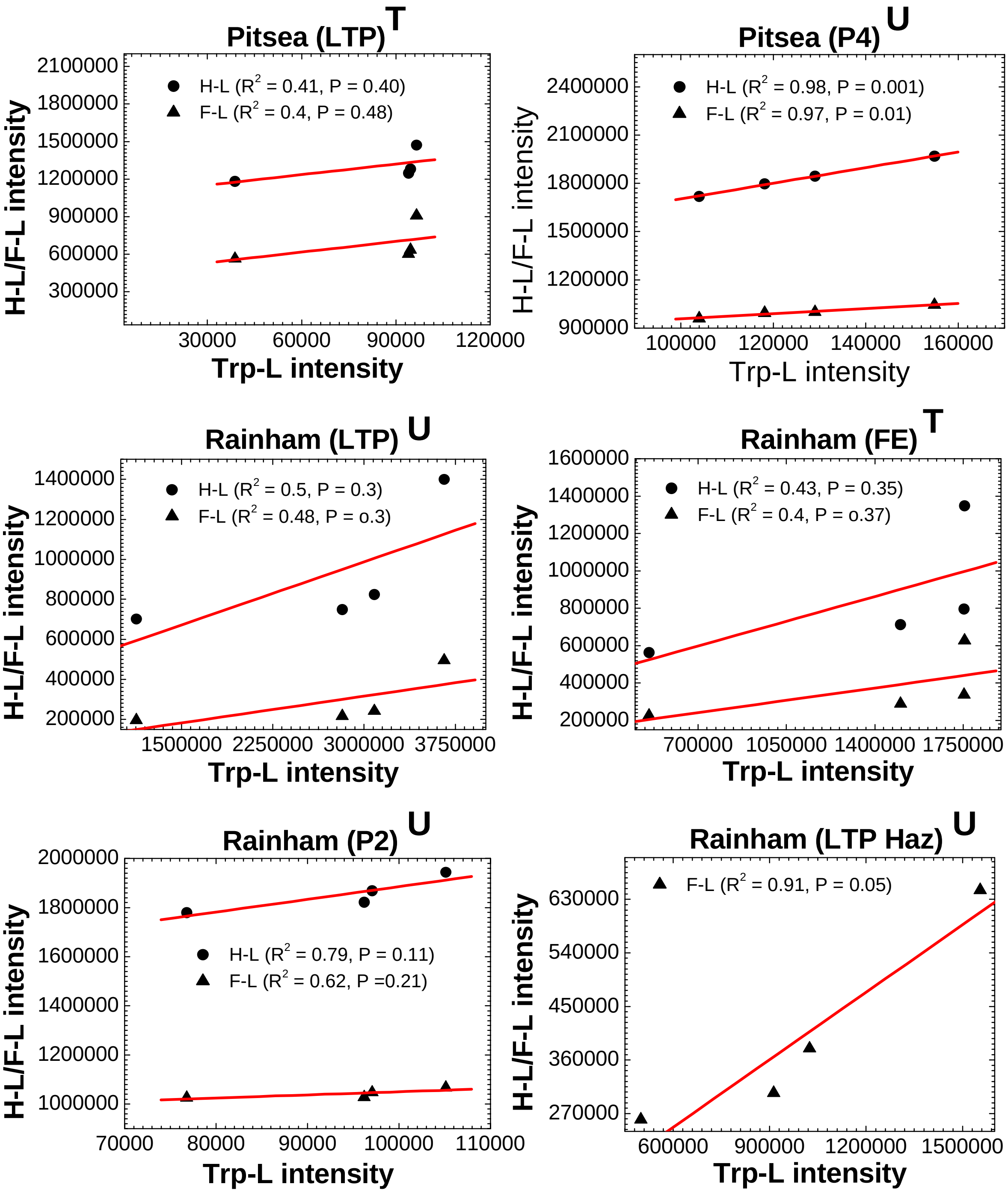


Figure 4.7 Comparisons between H-L/F-L and Trp-L (270-280 nm excitation) fluorescence intensity for; Pitsea (LTP), H-L: y = 1.07e6 + 2.84x, F-L: y = 444053 + 2.84x; Pitsea (P4), H-L: y = 1.22e6 + 4.58x, F-L: y = 797986 + 1.6x; Rainham (LTP), H-L: y = 356587 + 0.21x, F-L: y = 48426 + 0.09x; Rainham (FE), H-L: y = 332969 + 0.4x, F-L: y = 109036 + 0.2x; Rainham (P2), H-L: y = 1.4e6 + 5.2x, F-L: y = 923875 + 1.2x; Rainham (LTP Haz), F-L: y = 19888 + 0.4x. (P = probability that R = 0) (T = treated and U = untreated) (Note: non-zero axes are intercepts)

4.5.2 Relationship between fluorescence intensity and COD

The fluorescence intensities of H- L and F- L compounds are plotted against COD for all of the untreated and treated leachate samples in figure 4.8. Linear correlation coefficients are also shown. The results show that relationship between fluorescence intensity and COD varied among leachate samples. Leachate Rainham (P2) gave the best relation with $R^2 = 0.99$ and 0.98 for H- L and F- L fluorescence respectively, with the Rainham (LTP) leachate giving the weakest relation (H- L, $R^2 = 0.81$ and F- L, $R^2 = 0.79$). Intermediate values were found for Pitsea (LTP), Pitsea (P4), Rainham (LTP Haz) and Rainham (FE) leachates (figure 4.8).

Figure 4.8 shows that the fluorescence values decreased with decrease in COD for all of the leachates. Similar relations between COD and fluorescence intensities of H-L and protein-like peaks have been demonstrated by Bari and Farooq (1984), Reynolds and Ahmad (1997), Reynolds (2002), Baker and Curry (2004) and Fu et al. (2007).

4.5.3 Relationship between UV absorbance at 254 nm (UV_{254}) and COD

UV absorbance at 254 nm (UV_{254}) is plotted against COD for all of the untreated and treated leachate samples in figure 4.9. Linear correlation coefficients are also shown. Leachate Rainham (LTP) gave the best relation with $R^2 = 0.99$ with the Rainham (FE) leachate giving the weakest relation ($R^2 = 0.73$). Intermediate values were found for Pitsea (P4), Rainham (P2), Pitsea (LTP) and Rainham (LTP Haz) leachates (figure 4.9). These results indicate that chemical oxidation of organic matter might be associated with the splitting of unsaturated bonds and the dissociation of the aromatic rings and hence reduction of both COD and UV_{254}. Bari and Farooq (1984) have also demonstrated that strong correlations between COD and UV absorbance for wastewater.

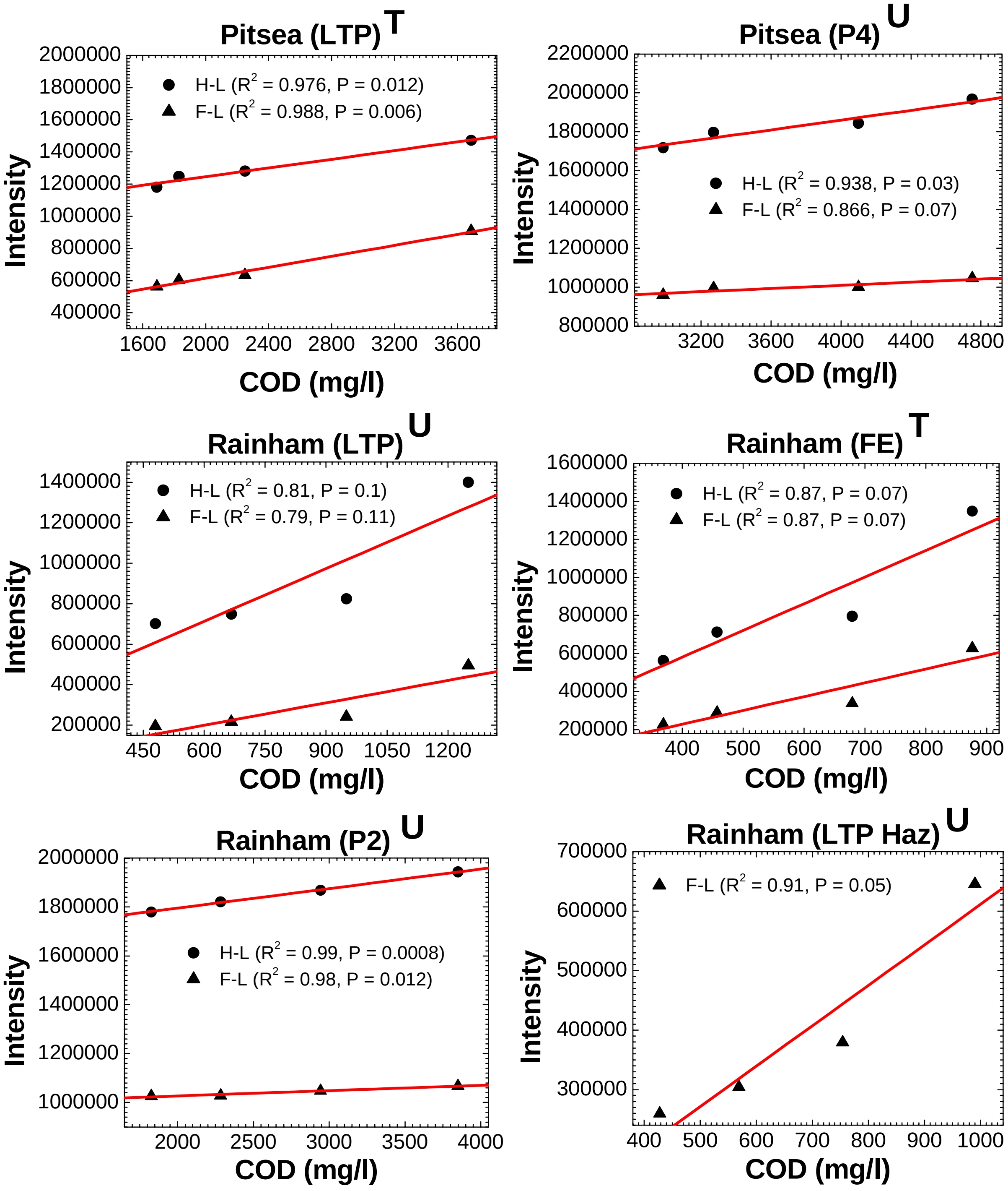


Figure 4.8 Comparisons of the fluorescence peaks with COD for; Pitsea (LTP), H-L: y = 975899 + 135x, F-L: y = 274116 + 170x; Pitsea (P4), H-L: y = 1.36e6 + 126x, F-L: y = 847724 + 40x; Rainham (LTP), H-L: y = 192965 + 867x, F-L: y = -21913 + 369x; Rainham (FE), H-L: y = 18876 + 1404x, F-L: y = -62497 + 726x; Rainham (P2), H-L: y = 1.64e6 + 80x, F-L: y = 983301 + 22x; Rainham (LTP Haz), F-L: y = -68567 + 680x. (P = probability that R = 0) (T = treated and U = untreated) (Note: non-zero axes are intercepts)

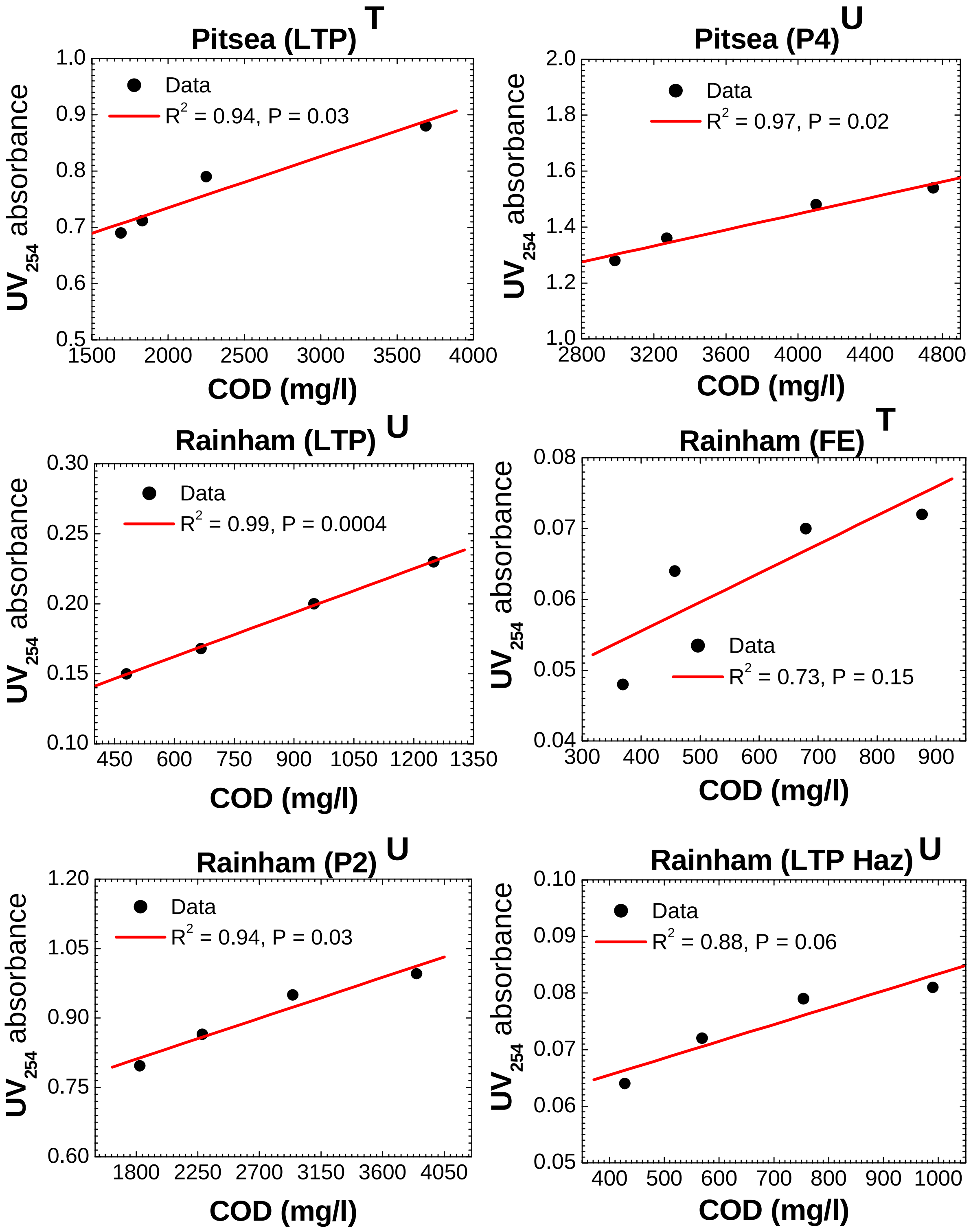


Figure 4.9 Comparisons of the UV_{254} with COD for; Pitsea (LTP), y = 0.6 + 9e-5x; Pitsea (P4), y = 0.9 + 1.4e-4x; Rainham (LTP), y = 0.1 + 1.1e-4x; Rainham (FE), y = 0.04 + 4.1e-5x; Rainham (P2), y = 0.6 + 9.8e-5x; Rainham (LTP Haz) y = 0.05 + 3e-5x. (P = probability that R = 0) (T = treated and U = untreated) (Note: non-zero axes are intercepts)

4.6 Summary

In this chapter the behaviour of organic compounds in leachates undergoing an aerobic biological treatment processes was investigated. Aerobic biodegradation was carried out over a period of 30 days using four untreated and two treated leachate samples collected from two UK MSW landfills, Pitsea and Rainham. Leachates were characterised using conventional methods (COD and DOC) as well as the potentially useful characterisation techniques of UV absorption and fluorescence spectroscopy. A good agreement was observed between the spectroscopic method and the well established COD and DOC methods of studying the biodegradation potential of different leachates. This established the fact that a rapid assessment of the biodegradation of leachates using spectroscopic method was in general reliable. In addition, the use of UV and fluorescence spectroscopy allowed some analysis of leachates and it was suggested that organic compounds in Pitsea and Rainham leachates were mostly aromatic in nature. Fluorescence spectroscopic result showed a higher percentage reduction of protein-like (Trp-L) compounds than humic (H-L) and fulvic (F-L) compounds during aeration confirming that protein-like compounds were mostly biodegradable whereas H-L and F-L compounds were the key components of the recalcitrant organic compounds. UV and the fluorescence spectroscopic analyses also suggested that leachates containing higher concentration of aromatic compounds and condensed aromatic structures are difficult to degrade. A combined analysis by novel UV and fluorescence spectroscopy and conventional methods indicated that Rainham (LTP) and Rainham (FE) is more easily biodegradable whereas Pitsea (LTP), Raniham (LTP Haz), Pitsea (P4) and Rainham (P2) are not so easily biodegradable.

Chapter 5

Anaerobic biodegradation of solid waste

5.1 Introduction

This chapter investigates the characteristics of recalcitrant organic compounds in leachates during anaerobic biodegradation of different wastes. In addition, a compositional analysis of the leachates generated from different waste samples is carried out to understand the nature of the recalcitrant precursors; humic and fulvic-like compounds and non-recalcitrant materials that are generated from different types of wastes. This research aims at fulfilling the research objective of understanding the influence of different types of waste on the generation of recalcitrant organic compounds and would be informative for waste management practices.

An anaerobic biodegradation experiment was carried out for four types of wastes, i.e., fresh waste (FW), composted waste (CW), newspaper waste (NW) and synthetic waste (SW) in the BMP test reactors. The experimental setup and sample preparation techniques are described in detail in section 3.3. The biodegradation processes took place over a period of 150 days at approximately 30^{o}C. Anaerobic biodegradation was characterized through the measurement of the volume of biogas produced, determination of the loss of cellulose, hemi-cellulose and lignin in the waste samples by the measurements of Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Digestible Lignin (described in section 3.4.8) at the beginning and at the end of the experiment. Leachates generated in the BMP test reactors were taken periodically and investigations were performed to determine the composition of the leachates generated during different stages of waste biodegradation. As in Chapter 4, these investigations were carried out using conventional methods (i.e., COD and DOC) and using potentially new technique of UV absorption and fluorescence spectroscopy.

5.2 Characterisation of waste biodegradation in BMP reactors

5.2.1 Initial waste characterisation

Table 5.1 presents the initial composition of the four types of solid wastes. Newspaper waste and composted waste contained highest (81%) and lowest (48%) percentages of total carbon respectively, whereas fresh waste and synthetic waste contained intermediate percentages of total carbon of around 55%. Newspaper waste was mainly composed of 51% cellulose, and 7.4% hemi-cellulose. These results agree with the reported values by Komillis and Ham (2003). It was also found that composted waste contained the lowest percentages of cellulose and hemi-cellulose of all of the wastes. This can be attributed to the fact that much of the readily biodegradable cellulose and hemi-cellulose of the original waste would have been degraded during composting. However, the percentage of lignin in composted waste and newspaper waste was higher than in fresh waste and synthetic waste. The hemi-cellulose and lignin contents of the fresh waste and composted waste found in this experiment were higher whereas cellulose content was lower than the values reported by Zheng et al. (2007) which might be due to the variation of the fibre measurements.

Figure 5.1 shows the cumulative biogas volume of fresh waste, composted waste, newspaper waste and synthetic waste corrected to STP. This figure also shows the cumulative biogas production in the control (blank) reactors which started to produce biogas from the beginning indicating the bacterial activity in these reactors. The control (blank) reactors containing the methanogenic mineral media and sewage sludge were used to determine the volume of biogas produced from the bacteria alone. The cumulative biogas volume at STP for the control reactors was 10.5 l/kg after 150 days of anaerobic biodegradation. The biogas produced by each test waste was determined by subtracting the biogas produced in the control reactor from the total biogas produced in each reactor. The cumulative biogas volume produced at STP for fresh waste, composted waste, newspaper waste and synthetic waste were 67.5 l/kg, 24 l/kg, 48 l/kg and 67 l/kg respectively after 150 days of anaerobic biodegradation. Although after 150 days, the cumulative gas production in fresh waste and synthetic waste was the same, the rate of mineralisation of organic matter in synthetic waste was slower than in fresh waste. This could be due to the initial lower nitrogen content of synthetic waste (Table 5.1) which might slow down the mineralisation of cellulose (Francou et al., 2008). Also the total gas production in composted waste and newspaper waste was lower than in fresh waste and synthetic waste, probably due to the higher lignin fraction in composted waste and newspaper waste samples (Table 5.1) and/or transfer into leachates. The low gas production in composted waste can also be attributed to the fact that much of the readily biodegradable carbon would have been degraded during composting. Almost 94% and 87% of the total biogas was produced within 70 days for composted waste and newspaper waste respectively. This indicates that, in these wastes, most of the bioavailable organic carbon was utilised within 70 days.

Table 5.1 Initial composition of solid waste components

Chemical analysis	**Fresh waste**	**Composted waste**	**Newspaper waste**	**Synthetic waste**	**Fresh waste (Zheng et al., 2007**	**Composted waste (Zheng et al., 2007**
Cellulose, %	25.0	12.0	51.0	30.0	48.6	32.8
Hemi-cellulose, %	9.5	6.0	7.5	12.0	7.4	2.8
Lignin, %	15.0	20.0	21.0	12.0	8.4	18.6
Total carbon (TC), %	56.0	48.0	81.0	54.0		
Total nitrogen (TN), %	2.2	2.5	1.9	1.8		

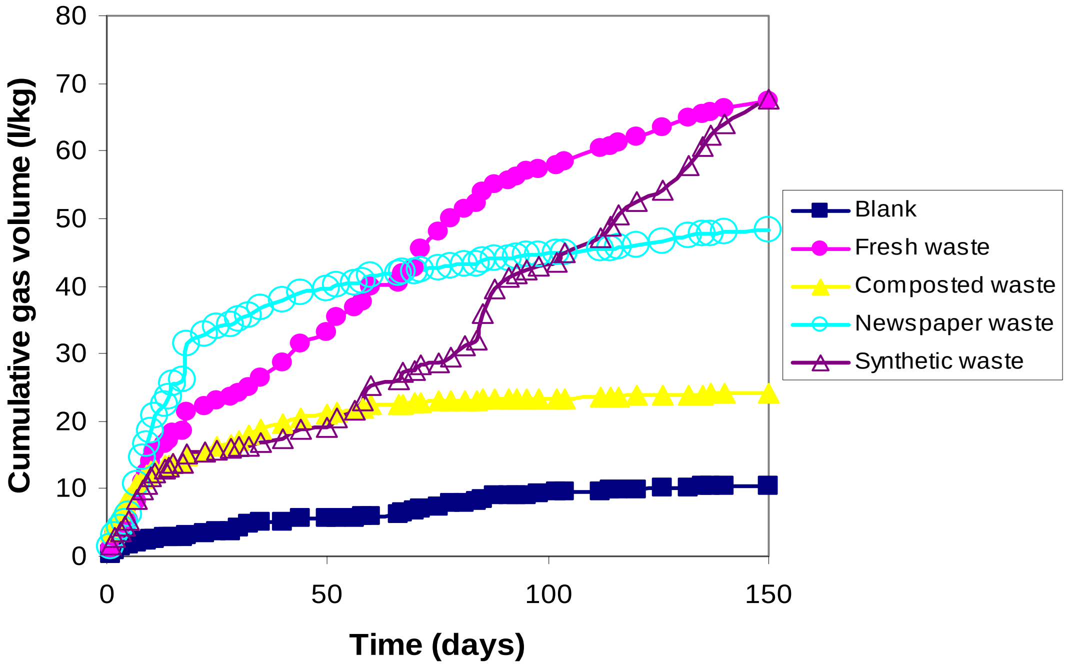
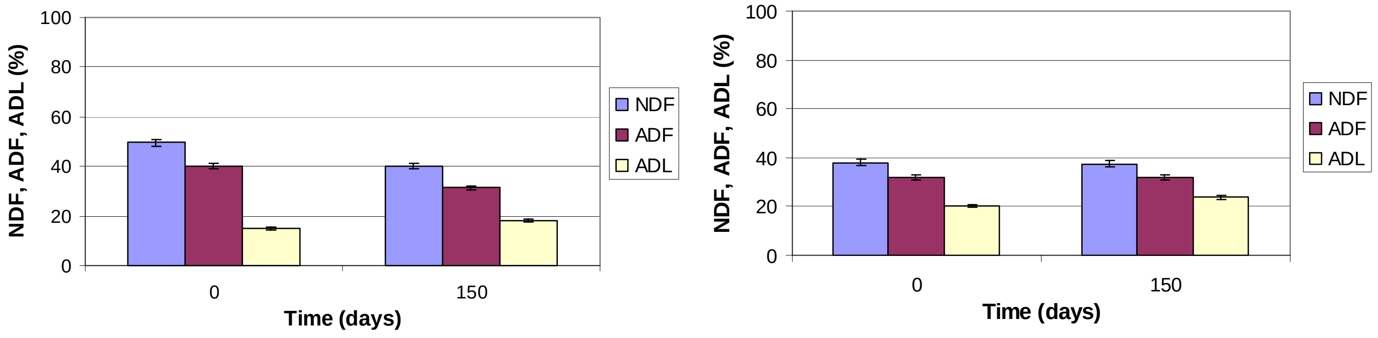


Figure 5.1 Cumulative gas productions at STP for all of the wastes and control (blank) in BMP reactors

5.2.2 Evolution of biochemical fractions during anaerobic biodegradation of wastes

The results of the NDF, ADF and ADL tests for all four types of waste samples at the beginning and at the end of the anaerobic biodegradation process are shown in figure 5.2 (a-d) (detailed description in section 3.3.4 and 3.4.8). The NDF content of the waste samples, which is indicative of the entire fibre fraction of the waste showed a decreasing trend over the test period for every waste sample indicating cellulose and hemi-cellulose degradation (figure 5.2 (a-d)). The NDF biodegradation rate was 0.61 g /kg DM/day, 0.04 g /kg DM/day, 1.63 g /kg DM/day and 0.47 g /kg DM/day for fresh waste, composted waste, newspaper waste and synthetic waste respectively by the end of 150 days. A similar decreasing trend was observed for ADF biodegradation for all of the waste samples (figure 5.2 (a-d)). ADF represents the portion of the waste that contains only cellulose and lignin. The average ADF biodegradation rates were 0.56 g /kg DM/day, 0.003 g /kg DM/day, 1.55 g /kg DM/day and 0.42 g /kg DM/day for fresh waste, composted waste, newspaper waste and synthetic waste respectively over the whole period of test. ADL represents the portion of the waste that contains only lignin. It is interesting to note that all of the four wastes showed a mild increase in the percentage of lignin fraction after 150 days of biodegradation (figure 5.2 (a-d)). The maximum increase was for synthetic waste (12.8% to 18%). The increase can be attributed to the low degradation of lignin and relatively high degradation of cellulose and hemi-cellulose over time thereby increasing the percentage of ADL. Lignin is known to be non-biodegradable/recalcitrant in anaerobic environments (Komilis and Ham, 2003).

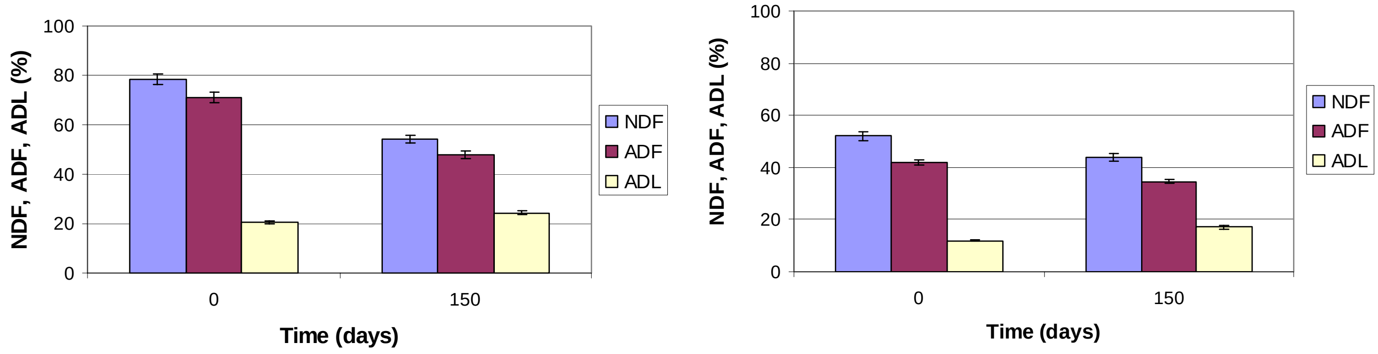


(a)

Fresh waste

(b)

Composted waste



(c)

Newspaper waste

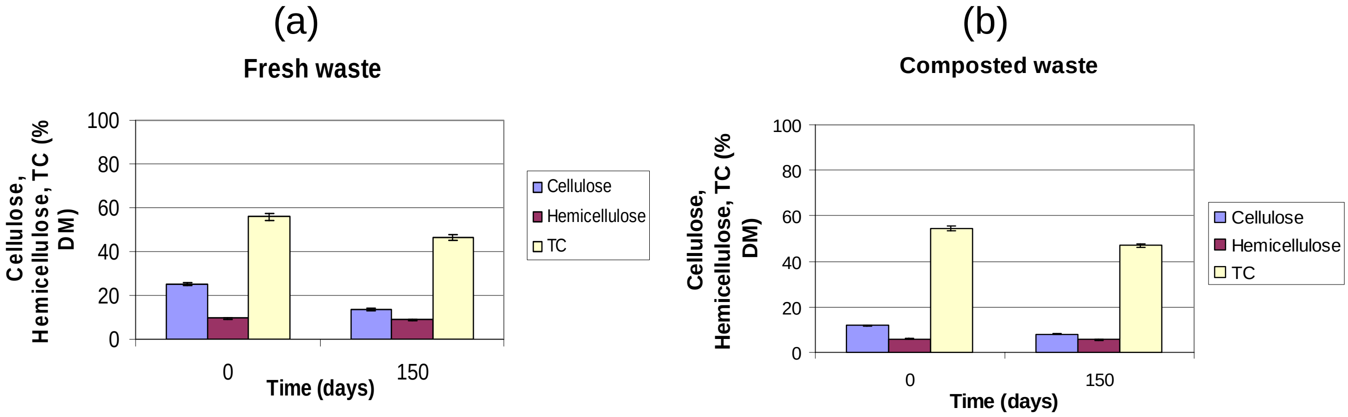
(d)

Synthetic waste

Figure 5.2 NDF, ADF and ADL over time for fresh waste, composted waste, newspaper waste and synthetic waste samples

Average values of cellulose and hemi-cellulose content for the four waste samples at the beginning and at the end of anaerobic biodegradation were estimated using Eq. 3.7 and Eq. 3.8 (Chapter 3), and are presented in figure 5.3 (a-d). The percentage of cellulose in the BMP reactors decreased over time at an average rate of 0.77 g /kg DM/day, 0.26 g /kg DM/day, 1.8 g /kg DM/day and 0.82 g /kg DM/day for fresh waste, composted waste, newspaper waste and synthetic waste respectively over the period of 150 days. Thus, 46%, 32%, 54%, 41% of the cellulose degraded for fresh waste, composted waste, newspaper waste and synthetic waste respectively. The highest percentage of cellulose degradation in newspaper waste can be attributed to the fact that cellulose found in paper is less resistant to biodegradation since much of its primary structure has already been destroyed during the pulping process (Micales and Skog, 1997). The comparatively lower percentages of cellulose degradation in fresh waste and synthetic waste than in newspaper waste were due to the fact that fresh waste and synthetic waste contained a low percentage of newspaper (35%) (Table 3.3). The lowest percentage of cellulose degradation in composted waste can be attributed to the fact that most of the readily biodegradable cellulose in the original waste would already have been degraded during composting, and the remaining cellulose might be very resistant to biodegradation. Hemi-cellulose degradation over the period of 150 days for fresh waste, composted waste, newspaper waste and synthetic waste was 10.5%, 8.3%, 18.7% and 8% respectively (figure 5.3 (a-d)); was less than the cellulose degradation. Thus cellulose is a better indication of degradation than hemi-cellulose under anaerobic conditions (Komilis and Ham, 2003).

Figure 5.3 (a-d) presents the total carbon (TC) fractions measured for all of the wastes at the beginning and end of anaerobic biodegradation process. It was found that TC content showed a decreasing trend over the test period for all of the waste samples. TC represents both organic and inorganic carbon fractions and cellulose constitutes a significant fraction of the initial TC in the waste (45% for fresh waste, 25% for composted waste, 63% for newspaper waste and 55% for synthetic waste). Therefore the decreasing trend of TC can be explained by the cellulose degradation which is consistent with the observed trend of cellulose.



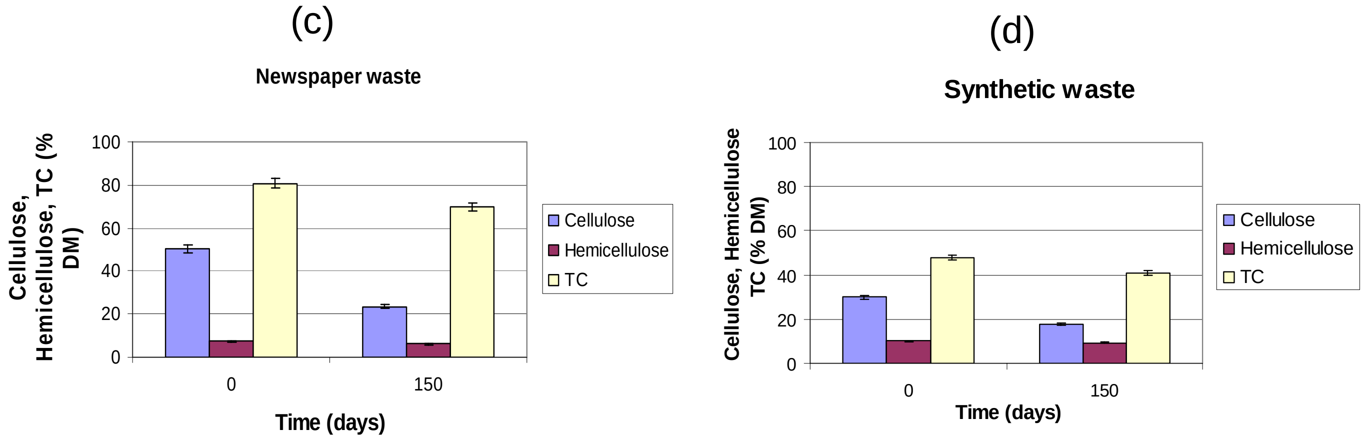


Figure 5.3 Cellulose, hemi-cellulose and TC over time for fresh waste, composted waste, newspaper waste and synthetic waste samples

The (Cellulose + Hemi-cellulose) to Lignin ratio, (C+H)/L may be used to demonstrate the extent of biodegradation or biodegradation potential (Wang et al., 1994). Figure 5.4 (a-d) presents (C+H)/L ratios for all of the wastes; a decreasing trend was observed. After 150 days of anaerobic biodegradation, the (C+H)/L values of fresh waste, newspaper waste and synthetic waste fell from 2.3, 2.81 and 3.5 to 1.23, 1.2 and 1.58 respectively, while the (C+H)/L ratio of the composted waste fell from 0.9 to 0.56. This decreasing trend could be explained by the fact that although lignin in the waste dry mass increased with time, the cellulose and hemi-cellulose (C+H) degradation was greater than the increase of lignin, resulting in a decrease of the (C+H)/L ratio (Wang et al., 1994; Hossain et al., 2003). The initial (C+H)/L ratios for fresh waste, newspaper waste and synthetic waste were between 2.0

and 4.0 which closely matches the values reported in the literature for fresh MSW (Bookter et al., 1982; Hossain et al., 2003). The low value of (C+H)/L for composted waste was consistent with the prior decomposition of waste.

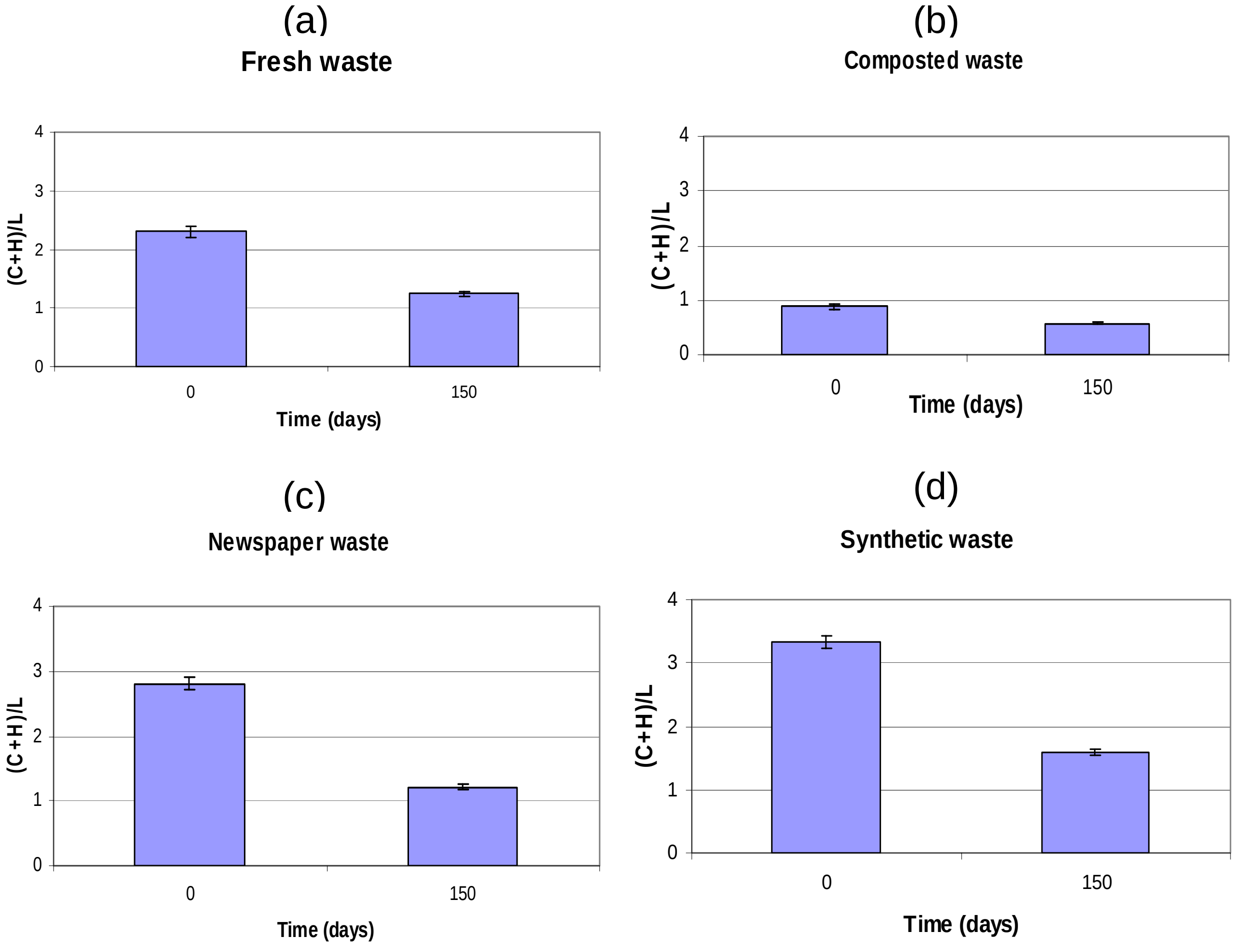


Figure 5.4 (C+H)/L over time for fresh waste, composted waste, newspaper waste and synthetic waste samples

5.3 Characterisation of leachates formed in BMP reactors

This section presents the chemical characteristics of leachates produced from the anaerobic biodegradation of all of the wastes in BMP reactors.

5.3.1 pH of leachate samples

The pH of the leachate samples generated from the waste biodegradation in BMP test reactors is shown in figure 5.5. Initially the pH of the leachates for fresh waste, composted waste, newspaper waste and synthetic waste were 7.1, 7.6, 7.2 and 7.3 respectively. These values decreased to minima pH of 4.9 (fresh waste), 5.0 (composted waste), 4.9 (newspaper waste) and 5.2 (synthetic waste) on day 10. This decrease in pH was probably due to the accumulation of volatile fatty acids (Chian and DeWalle, 1976). After day 20, a gradual increase in the pH value for all the wastes leachates was observed, to approximately level values of 7.1, 7.3, 7.2 and 7.0 for fresh waste, composted waste, newspaper waste and synthetic waste respectively over the last 80-100 days of the test.

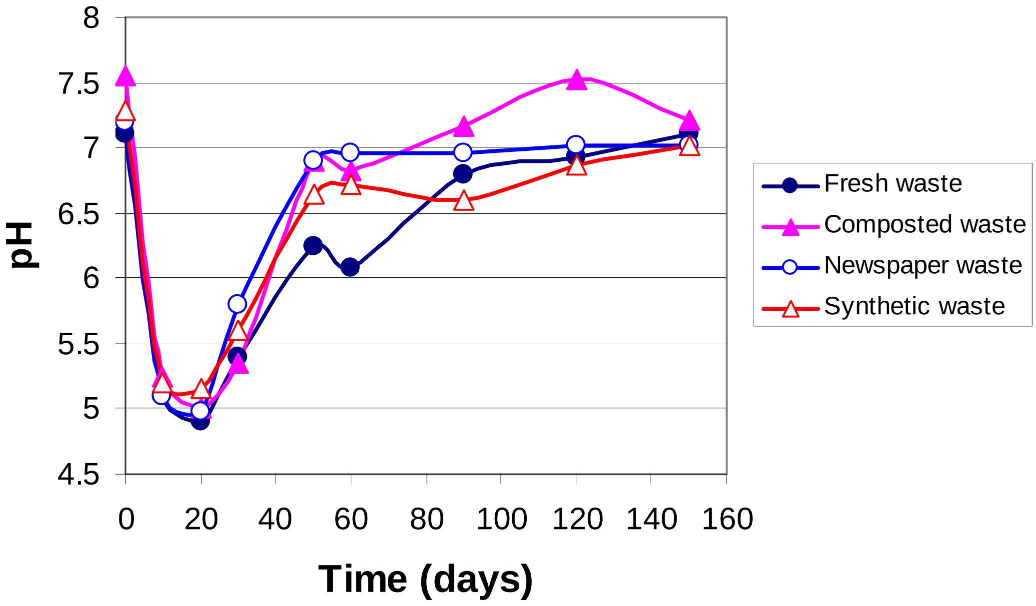


Figure 5.5 pH of leachates taken from BMP reactors

5.3.2 Carbon contents of leachate samples

Figure 5.6 (a-d) presents the total organic carbon (TOC), dissolved organic carbon (DOC), total inorganic carbon (TIC) and dissolved inorganic carbon (DIC) contents of leachate samples taken from the reactors of each waste sample at different stages of the biodegradation process. Figure 5.6 (a-d) shows that TOC content increased from an initial value of 1308 mg/l to 4625 mg/l after 35 days, 1813 mg/l after 5 days, 3888 mg/l after 25 days and 5468 mg/l after 22 days and then decreased gradually to levels of 705 mg/l, 176 mg/l, 1408 mg/l and 1160 mg/l for fresh waste, composted waste, newspaper waste and synthetic waste respectively by the end of the test. At the same time, the DOC content of the leachate samples (which had previously been filtered through 1.2 µm Whatman GF/C filters) increased from 422 mg/l to 4381 mg/l (day 25), 1452 mg/l (day 5), 3821 mg/l (day 22) and 4918 mg/l (day 22) and then decreased (showing the same pattern as TOC) to 412 mg/l, 180 mg/l, 1110 mg/l and 489 mg/l for fresh waste, composted waste, newspaper waste and synthetic waste respectively by the end of the test. The initial increase was most likely to have been due to the degradation of solid organic matter resulting in the formation of a range of soluble organic matter (Ivanova, 2007).

Figure 5.6 (a-d) also shows that the TIC content decreased from an initial value of 191 mg/l to 67 mg/l (5 days), 57 mg/l (5 days), 38 mg/l (10 days) and 68 mg/l (3 days) and then increased to levels of 128 mg/l, 556 mg/l, 182 mg/l and 328 mg/l for fresh waste, composted waste, newspaper waste and synthetic waste respectively by the end of the test. At the same time, the DIC content of the leachate samples decreased from 141 mg/l to 43 mg/l (day 5), 43 mg/l (day 3), 34 mg/l (day 5) and 44 mg/l (day 3) and then increased (showing the same pattern as TIC) to 112 mg/l, 476 mg/l, 169 mg/l and 306 mg/l for fresh waste, composted waste, newspaper waste and synthetic waste respectively by the end of the test. The initial decrease was most likely to have been due to the consumption of inorganic carbon by bacteria for cell growth (Ivanova, 2007).

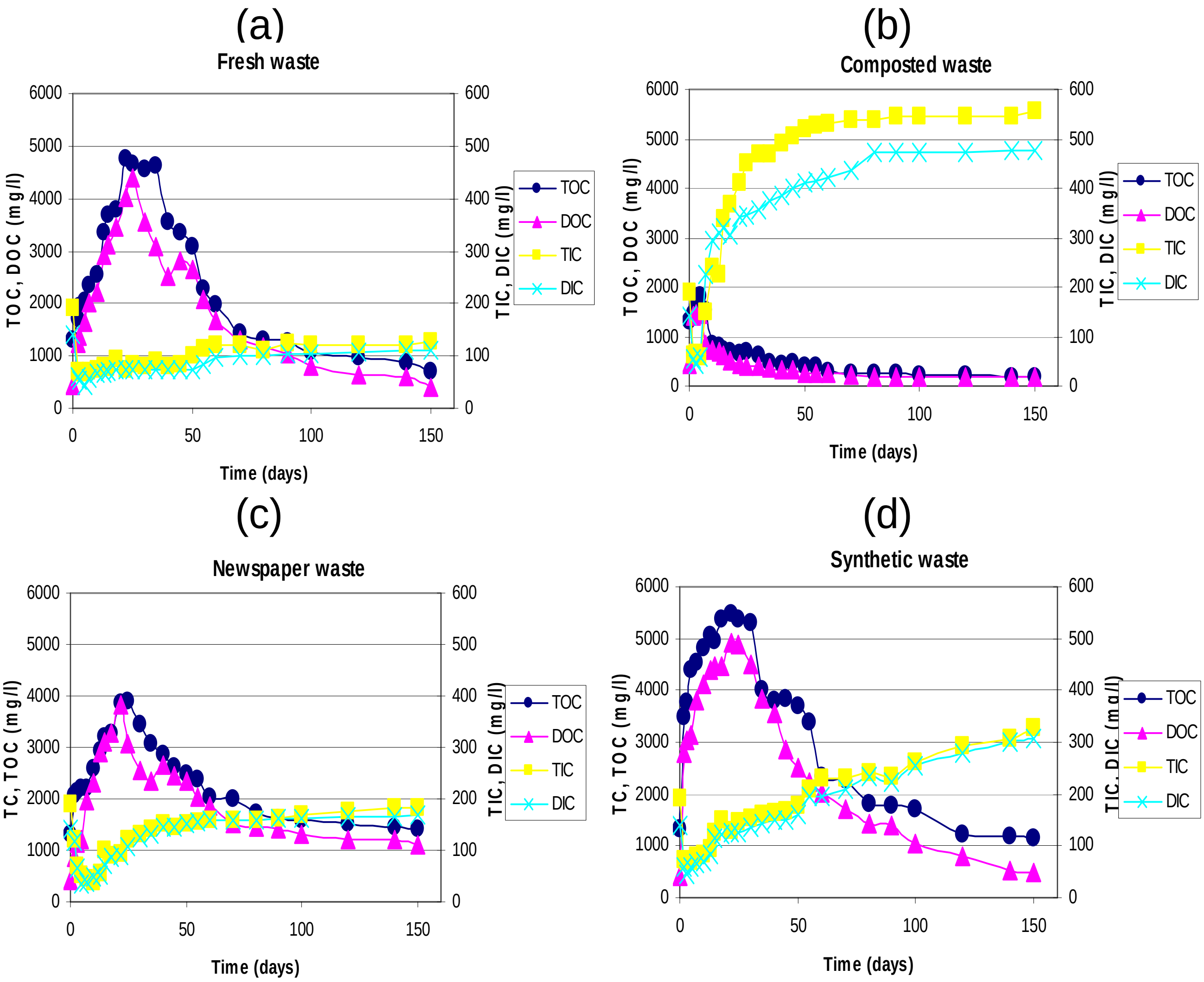


Figure 5.6 Variation of total and dissolved organic and inorganic carbon of leachates with time for fresh waste, composted waste, newspaper waste and synthetic waste samples (dilution factor 25, 50, 40 and 10 for fresh waste, composted waste, newspaper waste and synthetic waste respectively)

Figure 5.7 (a-d) presents the total chemical oxygen demand (TCOD) and dissolved chemical oxygen demand (DCOD) for leachate samples taken from the reactors of each waste sample at different stages of biodegradation. Figure 5.7 (a-d) shows that TCOD decreased from an initial value of 982 mg/l to 537 mg/l, 122 mg/l, 215 mg/l and 521 mg/l after 3 days, then increased gradually to levels of 2392 mg/l, 796 mg/l, 2056 mg/l and 1830 mg/l after 30 days and then again decreased to levels of 623 mg/l, 295 mg/l, 623 mg/l, 589 mg/l for fresh waste, composted waste, newspaper waste and synthetic waste respectively by the end of the test. At the same time, the DCOD of the leachate samples (which had previously been filtered through 1.2 µm Whatman GF/C filters) decreased from an initial value of 537 mg/l to 263 mg/l, 56 mg/l, 122 mg/l and 200 mg/l after 3 days, then increased gradually to levels of 2212 mg/l, 699 mg/l, 1816 mg/l and 1668 mg/l after 30 days and then again decreased (showing the same pattern as TCOD) to levels of 521 mg/l, 189 mg/l, 498mg/l, 498 mg/l for fresh waste, composted waste, newspaper waste and synthetic waste respectively by the end of the test. The initial decrease could be attributed to the consumption of organic matter by the bacteria. At the beginning some of the organic matter may be used for the bacterial growth, after that biodegradation started and the increase was likely to be due to the degradation of solid organic matter resulting in the formation of a range of soluble organic matter (Ivanova, 2007).

Figures 5.6 (a-d) and 5.7 (a-d) show that the organic and inorganic compounds in the leachates were mostly in dissolved form. However, these results also show that apart from the variation due to the rise and fall of organic and inorganic carbon, TOC, DOC TCOD and DCOD values for fresh waste, newspaper waste and synthetic waste leachates were significantly higher than for composted waste leachates whereas the opposite trend was true for inorganic carbon content. This could certainly be attributed to the fact that for composted waste, a large amount of the biodegradable organic carbon present in the original waste would have been degraded during composting. It was also found that a considerable amount of organic compounds remained to be removed through biodegradation in fresh waste, newspaper waste and synthetic waste leachates. To gain a better understanding, TCOD is plotted against TOC for all of the wastes' leachates and is presented in figure 5.8 (a-d). The good relationship observed in the fresh waste, newspaper waste and synthetic waste leachates could be attributed to the presence of a significant amount of organic compounds in the leachates which was progressively mineralized. As can be seen the value of R^2 for composted waste leachates was weak ($R^2 = 0.15$). This probably indicates that composted waste contained very little readily biodegradable organic carbon.

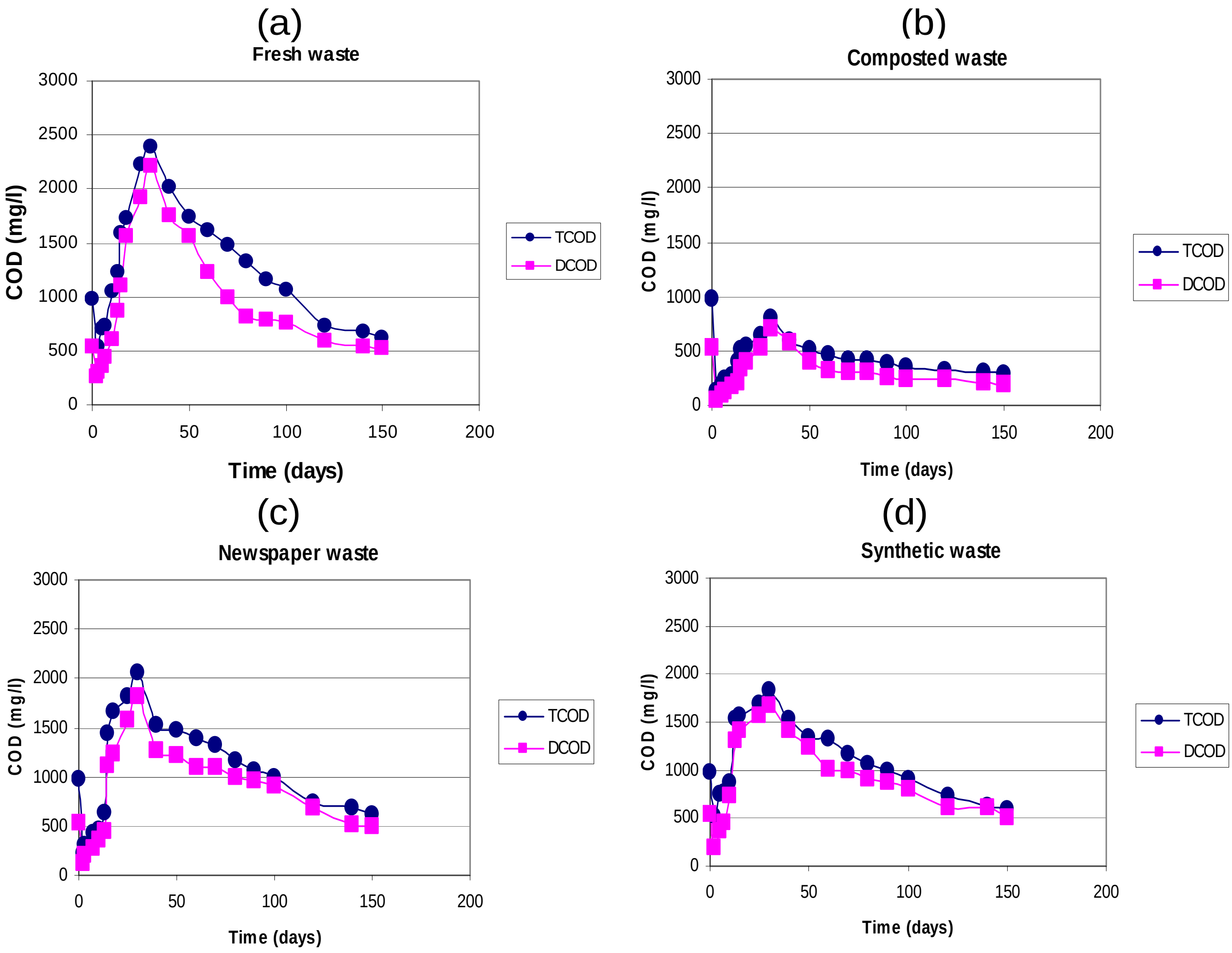


Figure 5.7 Variation of total and dissolved COD of leachates with time for fresh waste, composted waste, newspaper waste and synthetic waste samples (dilution factor 25, 50, 40 and 10 for fresh waste, composted waste, newspaper waste and synthetic waste respectively)

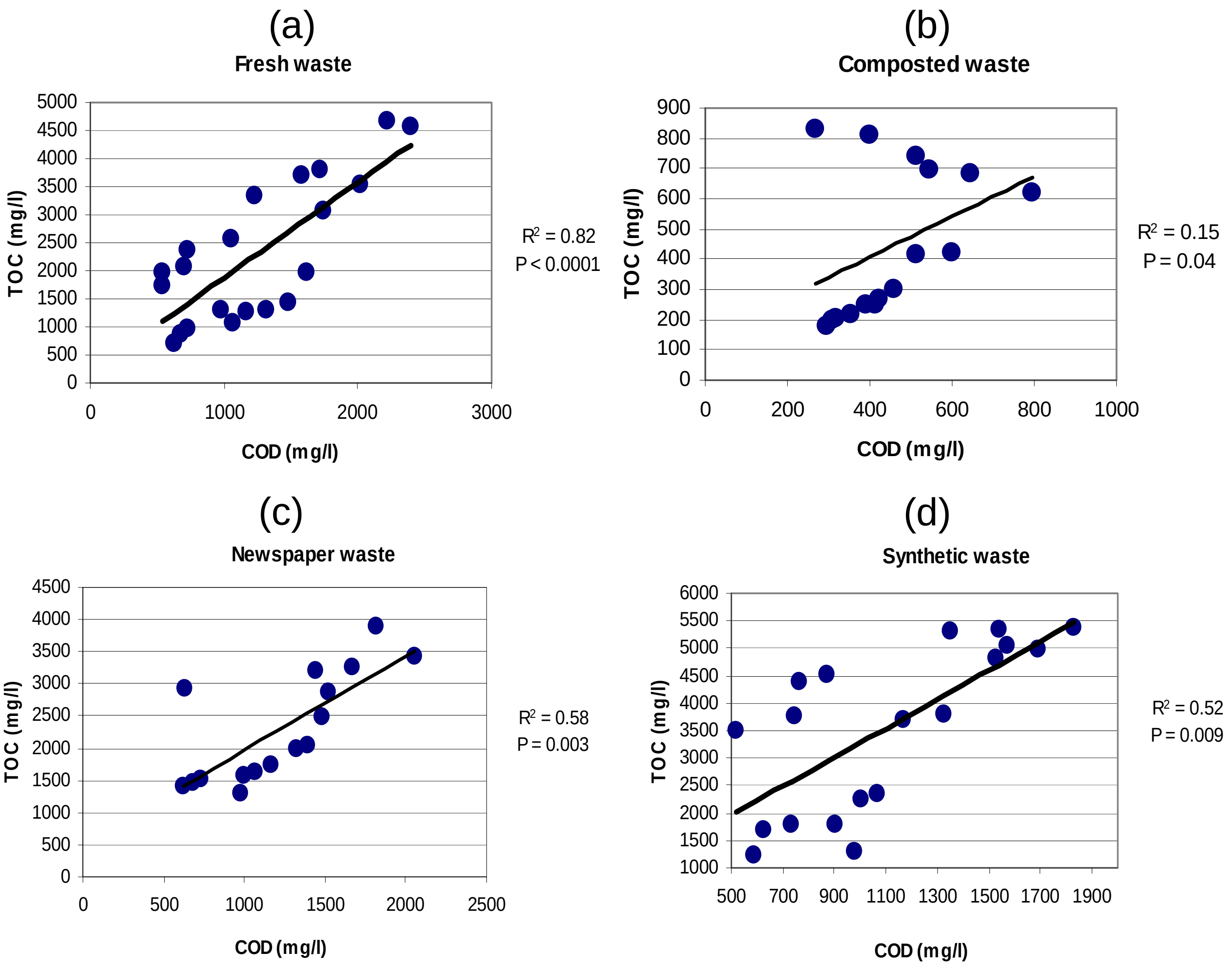


Figure 5.8 Comparisons of TOC with COD of leachates taken from fresh waste, composted waste, newspaper waste and synthetic waste samples (P = probability that R = 0)

5.4 Mass balance estimate for the BMP reactors

A mass balance estimate was undertaken to monitor the carbon transfer from the solid to the liquid and gas phases for all four wastes. After 150 days, all of the BMP bottles were emptied and samples from the waste and leachate were taken for total carbon analysis. The composition of the biogas produced (methane and carbon dioxide) from all of the wastes was investigated at the end of the biodegradation process and presented in Table 5.2. The loss of carbon by deposition of carbonate precipitates was estimated using the reductions in Ca^{+2} and Mg^{+2} concentrations between the beginning and the end of the test. The summary of carbon mass balance for waste samples is presented in Table 5.3. Table 5.3 indicates that 91 g, 81 g, 112 g and 71 g of carbon per kg dry wt. were utilized by fresh waste, composted waste, newspaper waste and synthetic waste respectively during the period of biodegradation. It was found that 8.5% (fresh waste), 7.0% (composted waste), 10.5% (newspaper waste) and 15% (synthetic waste) of utilized TC was transferred into the leachate and 39% (fresh waste), 15% (composted waste), 22% (newspaper waste) and 48% (synthetic waste) into the biogas by the end of 150-day monitoring period.

The carbon mass balance estimates made for the BMP reactors indicate that the carbon input exceeds carbon output by 9.8%, 14.4%, 10.3% and 8.2% for fresh waste, composted waste, newspaper waste and synthetic waste respectively (Table 5.2). However, there are number of potential sources of error in the carbon mass balances, the most important of which are the neglect of any carbon utilized for bacterial biomass and growth; and random errors due to limitations in the measurements devices used in the experiment and the initial sampling.

Table 5.2 Composition of biogas produced from all of the wastes (methane and carbon dioxide)

Percentage volume of the total biogas produced	**Fresh waste**	**Composted waste**	**Newspaper waste**	**Synthetic waste**
Methane (CH_4)	60	70	67	60
Carbon dioxide (CO_2)	38	27	28	35

Table 5.3 Summary of carbon mass balance estimates for waste samples

Parameter	Fresh waste	Composted waste	Newspaper waste	Synthetic waste
$TC_{t=0,\ waste}$, g/kg DM	560	480	810	540
$TC_{t=0,\ leachate}$, g/kg DM	10.5	10.5	10.5	10.5
$TC_{t=ti,\ waste}$, g/kg DM	469	399	698	459
$TC_{t=ti,\ leachate}$, g/kg DM	7.5	5.7	11.8	10.4
$TC_{t=ti,\ CaCO3}$, g/kg DM	1.1	1.65	1.16	1.22
$TC_{t=ti,\ MgCO3}$, g/kg DM	0.4	0.45	0.61	0.6
$TC_{t=ti,\ CH4}$, g/kg DM	21.9	9.0	17.5	21.6
$TC_{t=ti,\ CO2}$, g/kg DM	13.8	3.5	7.3	12.7
Unaccounted C, g/kg DM	56.8	71.2	84	44.9
Mass balance error, %	9.8	14.4	10.3	8.2

Note: DM = dry matter; $TC_{t=0,\ waste}$ indicates the total carbon in the wastes before the beginning of the test; $TC_{t=ti,\ waste}$ indicates the total carbon in the wastes after 150 days of the test; $TC_{t=0,\ leachate}$ and $TC_{t=ti,\ leachate}$ are the TC content (TOC+TIC) of leachate samples taken at the beginning and at the end of the biodegradation respectively; $TC_{t=ti,\ CaCO3}$ and $TC_{t=ti,\ MgCO3}$ are the carbon contents as $CaCO_3$ and $MgCO_3$ in the leachate between the beginning and the end of the biodegradation respectively; $TC_{t=ti,\ CH4,}$ and $TC_{t=ti,\ CO2}$ are the carbon in the biogas considering methane and carbon dioxide respectively at the end of the test.

The results described in section 5.2 and 5.3 indicate that waste biodegradation and the characteristics of the leachates generated varies significantly with the nature and composition of wastes. For example, synthetic waste was prepared according to the composition of fresh waste (food, grass and paper) but the mineralisation of organic compounds was different in these fresh and synthetic wastes. It was also indicated that the total carbon transferred to leachate and gas was relatively low in composted waste (Table 5.3) as the readily biodegradable organic carbon had already been removed during composting and the remaining organic carbon was very resistant to anaerobic biodegradation. The biochemical composition and the gas production trends also showed that fresh waste and synthetic waste had the highest biodegradation potential of the other three wastes under consideration. Again, the high percentage of cellulose and lignin also seem to affect the biodegradation of newspaper waste. Therefore, it can be concluded that the composition and characteristics of leachates produced during the biodegradation not only depend on the nature of wastes but also on the anaerobic biodegradability of the waste components. It indicates that these factors might play an important role in the characteristics of recalcitrant organic compounds in leachates and hence it is important to investigate the evolution of these compounds in the leachates associated with the anaerobic biodegradation of wastes. This is presented in section 5.5.

5.5 Spectroscopic analysis

In this section the recalcitrant organic compounds in the leachates generated from different types of waste in the BMP reactors was studied using fluorescence and UV absorbance techniques. From TOC and COD analyses (section 5.2.2 and figures 5.6 and 5.7) it was found that organic matter in the leachate samples was mostly in dissolved form. Therefore the unfiltered leachate samples were analysed for their spectroscopic characteristics to obtain the representative information.

5.5.1 Fluorescence spectroscopic results

5.5.1.1 Excitation-Emission-Matrix (EEM) spectra of control samples

Figure 5.9 (a-c) shows 3D EEM spectra generated from fluorescence analyses of samples taken from control (blank) reactors containing anaerobic seed (section 3.3.2) and methanogenic nutrient media (Table 3.5) at days 5, 10 and 150 of the anaerobic biodegradation process (EEM spectra of the other days' samples are presented in Appendix B). The nutrient media before seed addition (before biodegradation started) showed no fluorescence (figure 5.9 (d)). However, in figure 5.9 (a-c), two apparent peaks were observed that excited at 220-230/270-280 nm and emitted at 300-370 nm for all days. The tryptophan-like (Trp-L) fluorophore refers to fluorescence centres at 220-230 nm and 270-280 nm excitation and 340-370 nm emission wavelengths (Baker et al., 2004; Elliot et al., 2006), whereas the tyrosine-like (Tyr-L) fluorescence centres excite at approximately 220-230 nm and 270-280 nm and emit at 300-320 nm wavelengths (Coble, 1996; Elliot et al., 2006). Therefore, these peaks observed in figure 5.9 (a-c) can be attributed to the tyrosine-like (Tyr-L) and tryptophan-like (Trp-L) compounds. These figures also show that the Tyr-L fluorescence at 270-280 nm excitation wavelengths was obscured by the Raman line of water and hence was ignored. These 3D EEM spectra suggest that both Tyr-L and Trp-L fluorescence might be attributed to the protein-like fluorescence peaks, which were directly related to the microbial activity of the anaerobic seed in the control reactors. Similar peaks have also been observed in the river water samples owing to the activity of planktonic bacteria (Elliot et al., 2006). However, the presence of the Tyr-L and Trp-L fluorescence peaks ascertained that anaerobic seeds were the possible cause of these ‘protein-like’ peaks observed in these control samples.

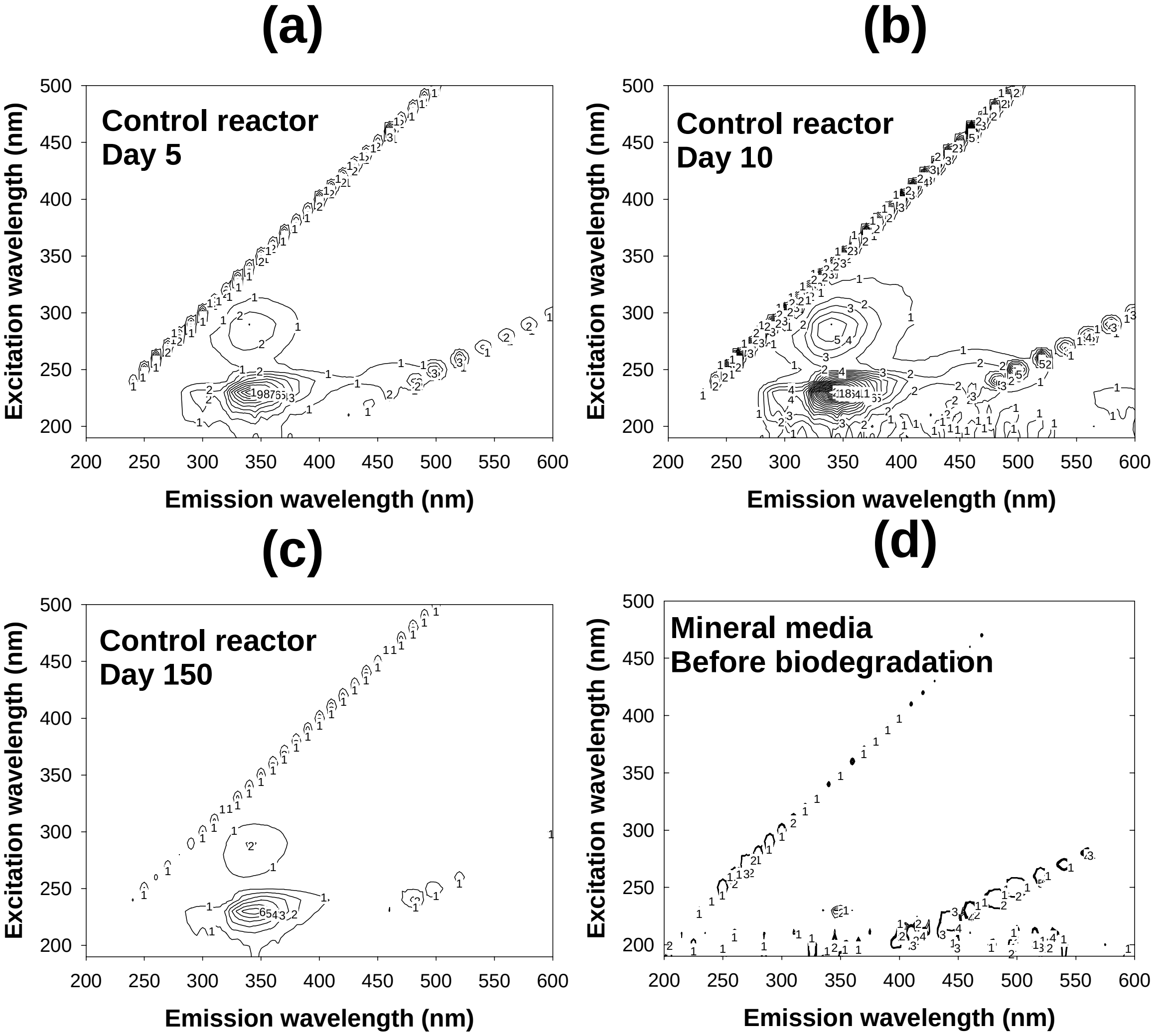


Figure 5.9 3D EEM fluorescence spectra of control (blank) reactors at day (a) 5, (b) 10 and (c) 150 showing Tyr-L and Trp-L fluorescence; (d) methanogenic mineral media at day 0 (dilution factor 25)

The changes in intensity of the Tyr-L and Trp-L fluorescence of the control (blank) samples over time are presented in figure 5.10. It was observed that the fluorescence intensities of these two fluorophores increased from day 5 to day 10 and then gradually decreased up to the end of the biodegradation process. This could be explained by the fact that the growth of the anaerobic seeds might be continuing up to day 10, giving the highest peaks at that time. After that, the numbers of cells decreased and a lower amount of proteins-like substances was produced over time (assuming that the protein-like substances produced were relative to the numbers of cells present (Elliot et al., 2006)). This could explain the decreasing trend of the intensity of Tyr-L and Trp-L fluorescence over time in control reactors.

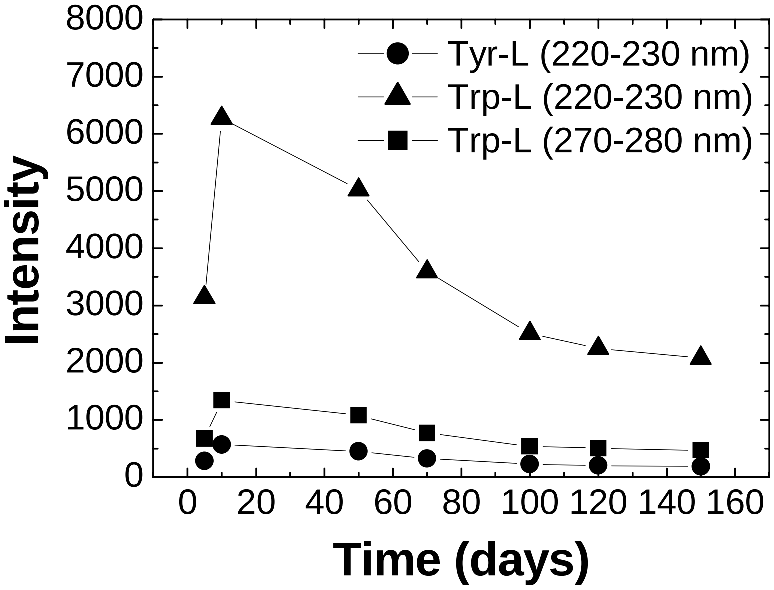


Figure 5.10 Variation of Tyr-L and Trp-L fluorescence intensity over time in the control (blank) reactor

5.5.1.2 Excitation-Emission-Matrix (EEM) spectra of leachate generated from all waste reactors

Table 5.4 presents the synthesis and/or utilization of the integrated fluorescence intensities and position of the EEM peaks of leachates taken at different days of the biodegradation process from fresh waste, composted waste, newspaper waste and synthetic waste reactors. In addition to the Tyr-L and Trp-L peaks, two other distinct zones were also identified on the EEM spectra (shown later) which were indicative of the presence of two types of organic substances.

Zone 1: A peak was observed in all of the samples between 230-260 nm and 370-390 nm excitations and 400-460 nm and 460-480 nm emission respectively and is widely recognized as a component of the humic-like fractions (H-L) (Coble, 1996; Baker and Curry, 2004; Cumberland and Baker, 2007).

Zone 2: Another peak was also present in all of the samples between 320-340 nm excitation and 400-440 nm emission, which can be attributed to aromatic and aliphatic groups in the DOM fractions and is commonly labelled as fulvic-like fractions (F-L) (Coble, 1996; Baker and Curry, 2004).

It is worth mentioning that the presence of H-L and F-L fluorescence was not observed until day 50 for leachates generated from all wastes. This might be attributed to the presence of low molecular weight biodegradable organic compounds in leachates due to the waste degradation during this period, which was also evidenced by the high values of TOC and COD (figures 5.6 and 5.7). As discussed in Chapter 2, H-L and F-L compounds are the essential building block of recalcitrant organic compounds. Therefore, relative comparison of the fluorescence peak intensities and peak positioning of H-L and F-L compounds of leachates generated from different types of wastes may reveal information on the dependence of the nature and composition of wastes on the evolution and characteristics of the recalcitrant organic compounds.

Table 5.4 Intensities and position of the EEM peaks for leachates taken from fresh waste, composted waste, newspaper waste and synthetic waste reactors (dilution factor 25, 50, 40 and 10 for fresh waste (FW), composted waste (CW), newspaper waste (NW) and synthetic waste (SW) respectively) (SD = standard deviation, n = 3)

Sample	Day	Zone 1 (H-L)				H-L intensity (column 4+column 6)	Zone 2 (F-L)		Tyr-L		Trp-L	
		Position (ex/em)	Intensity Mean/SD	Position (ex/em)	Intensity Mean/SD		Position (ex/em)	Intensity Mean/SD	Position (ex/em)	Intensity Mean/SD	Position (ex/em)	Intensity Mean/SD
FW	50	230-260/400-450	3294/7.6	370-390/460-480	1711/6.5	5005	310-350/400-440	9492/5.4			225-240/330-370	1451/5.5
	60	230-260/400-450	2967/9.5	370-390/460-480	1322/4.4	4289	310-350/400-440	8134/4.4			225-240/330-370	1511/5.0
	70	235-260/400-450	2224/4.5	370-390/460-480	1065/3.0	3289	310-350/400-440	6281/8.4			220-240/330-370	1718/6.4
	80	230-270/400-470	2545/5.0	370-390/460-480	1087/4.8	3632	310-350/400-440	7033/10.0			220-240/330-370	1799/7.4
	90	230-270/400-470	3056/8.6	370-400/470-490	900/7.0	3956	310-360/400-450	7999/9.8			230-240/330-370	198710.0
	100	230-270/400-470	4589/8.5	370-400/470-490	1504/5.5	6093	310-360/400-450	8322/5.8			230-240/330-370	20919.4
	120	220-260/400-450	4959/9.5	370-400/470-500	1211/3.8	6170	310-350/400-450	8136/10.0			240,270-280/350-370	533/8.0
	140	230-260/400-440	5234/5.0	370-395/470-500	1289/4.5	6523	300-355/400-450	7378/6.5			240,270-280/350-370	511/5.5
	150	230-260/400-440	5574/6.5	370-395/470-500	1483/5.2	7057	300-355/400-450	7197/6.6			240,270-280/350-370	434/10
CW	50	230-260/435-460	7323/10.8	350-360/450-490	791/8.2	8114	300-355/400-450	9410/7.4	270-280/305-320	911/5.5	230-240/330-400, 270-280/340-370	8400/8.4
	60	230-260/435-460	7634/6.5	350-360/450-490	6745.8	8308	300-355/400-450	10218/5.8	270-280/305-320	902/10.4	230-240/330-400, 270-280/340-370	7934/4.8
	80	230-260/440-460	7699/10.0	350-360/450-490	935/6.4	8634	300-355/400-450	10624/3.4	270-280/305-320	9146.8	235-240/330-400, 270-280/340-370	7646/5.4
	90	235-260/435-460	7974/10.0	370-380/470-500	1000/3.2	8974	300-355/400-450	9930/5.2	270-280/305-320	899/7.4	230-240/330-400, 270-280/340-370	7344/6.0
	100	230-260/435-460	10500/6.8	370-380/470-500	2002/4.2	12502	300-355/400-450	8852/9.8	270-280/305-320	905/4.5	235-240/330-400, 270-280/340-370	7155/3.8
	120	230-260/435-460	10539/6.6	365-375/470-490	2041/2.8	12580	300-355/400-450	8624/4.5	270-280/305-320	832/7.6	230-240/330-400, 270-280/340-370	6780/9.5
	140	230-260/435-460	10400/7.4	365-375/470-490	2342/5.5	12742	300-355/400-450	8246/8.0	270-280/305-320	801/5.8	230-240/330-400, 270-280/340-370	6156/7.4
	150	230-260/435-460	10890/7.6	365-375/470-490	1920/8.0	12810	300-355/400-450	8064/4.5	270-280/305-320	709/6.0	235-240/330-400, 270-280/340-370	5987/5.5

Table 5.4 (Contd.) Intensities and position of the EEM peaks for leachates taken from fresh waste, composted waste, newspaper waste and synthetic waste reactors (dilution factor 25, 50, 40 and 10 for fresh waste (FW), composted waste (CW), newspaper waste (NW) and synthetic waste (SW) respectively) (SD = standard deviation, n = 3)

Sample	Day	Zone 1 (H-L)				H-L intensity (column 4+column 6)	Zone 2 (F-L)		Tyr-L		Trp-L	
		Position (ex/em)	Intensity Mean/SD	Position (ex/em)	Intensity Mean/SD		Position (ex/em)	Intensity Mean/SD	Position (ex/em)	Intensity Mean/SD	Position (ex/em)	Intensity Mean/SD
NW	50						320-370/400-450	7452/4.6			270-280/350-370	605/7.4
	70	230-260/400-440	5130/10.0	370-390/460-480	1682/6.4	6812	300-340/400-440	8414/6.3	270-280/305-320	199/7.3	220-240/330-360	2489/6.4
	90	230-260/400-445	5547/5.8	370-395/460-480	1688/9.5	7235	300-340/400-440	8406/6.3			220-240/330-360	822/6.5
	100	230-255/400-445	6761/4.9	370-395/470-500	1470/4.7	8231	310-360/400-450	8423/5.5			270-280/355-370	549/7.8
	120	230-260/400-445	7031/6.8	370-395/470-500	1505/5.8	8535	310-360/400-450	8550/5.9			270-280/355-370	479/7.2
	140	230-265/400-445	7818/5.8	370-405/470-500	1863/5.8	9681	310-360/400-450	9185/6.2			235-240/350-370, 270-280/350-370	449/6.8
	150	230-265/400-445	8196/10.4	370-400/470-500	2123/6.8	10319	300-355/400-450	9245/5.4			235-240/350-370, 270-280/350-370	426/6.3
SW	50						270-310/390-440	2449/5.6				
	70						300-350/390-440	3807/7.3			270-280/350-380	
	90						265-315/390-450	3604/7.4			230-245/350-370	
	100						265-315/390-450	3202/6.5			230-245/345-370	791/6.6
	120						265-315/390-450	3600/6.8			230-245/345-370	921/7.6
	140						265-320/370-460	4409/6.6			230-245/340-370	916/7.7
	150						265-320/370-460	5524/5.4			230-245/340-370	1320/5.5

5.5.1.2.1 Comparative analyses of Excitation-Emission-Matrix (EEM) spectra of leachates generated from all wastes

Figures 5.11 to 5.14 show the EEM spectra of the leachate samples taken from the fresh waste, composted waste, newspaper waste and synthetic waste reactors respectively at days 50 and 150 of the biodegradation process (EEM spectra of the other days' samples are presented in Appendix B). These figures illustrate the presence of different peaks observed in leachates. The control corrected EEM spectra are also presented in these figures (assuming that microbial activity of the anaerobic seeds is similar in the control reactors as well as in the fresh waste, composted waste, newspaper waste and synthetic waste reactors).

Table 5.4 show that for fresh waste leachates, the intensities of H-L and F-L compounds decreased from day 50 to day 70. This decrease could be explained by the release of proteins into the leachates and their subsequent utilisation. In fresh waste leachate a high content of protein is expected due the presence of significant amount of food and grass in fresh waste (65%, Table 3.3). Protein usually contains methylene (-CH_2) functional groups which contributes to lipophilicity and it has been reported that the presence of lipophilic extractives could favour absorbing H-L and F-L compounds (Wu, 2002; Chen, 2003). Therefore, the intensity decrease of H-L and F-L compounds from day 50 to day 70 can be explained by the fact that a considerable amount of generated H-L and F-L compounds remains undetected by the fluorescence spectroscopy due to the sorption by lipophilic extractives present in the fresh waste leachate. However, this decrease of H-L/F-L fluorescence intensity was not observed in newspaper waste and synthetic waste leachates (Table 5.4). This could be attributed to the higher cellulose content of newspaper waste and synthetic waste compared to fresh waste (Chen, 2003). It is known that cellulose and hemi-cellulose contribute little to the overall sorption capacity as lipophilic extractives are small in these polymers (Chen, 2003). However, composted waste contained a lower percentage of cellulose and hemi-cellulose and a higher percentage of lignin than fresh waste, which could contribute towards the sorption of organics (Table 5.1). Nevertheless, a decrease of H-L/F-L fluorescence intensity was not observed in this leachate. This might be due to the fact that the sorption has been compensated by a significant H-L and F-L compounds generation during anaerobic biodegradation of composted waste.

It was also observed from figures 5.12 (a-b) and Table 5.4 that Zone 1 (H-L) fluorophores of composted waste leachates were in the long emission wavelengths. This suggested that H-L fluorophores of composted waste leachates were more aromatic and high molecular weight than those of the other leachates (Hudson et al., 2007). In composted waste, readily biodegradable organic carbon would have been degraded during composting. As a result, subsequent anaerobic biodegradation of the remaining organic matter would result in more biologically resistant H-L compounds which might be aromatic in nature. This explains the shift of H-L fluorophores to the longer emission wavelengths in this leachate. In addition, figure 5.14 (a-b) showed that in comparison to other leachates, Zone 2 (F-L) fluorophores of synthetic waste leachates were at shorter excitation and emission wavelengths. This indicated that Zone 2 (F-L) fluorophores of synthetic waste leachates might contain a lower proportion of aromatic rings than fresh waste, composted waste and newspaper waste leachates.

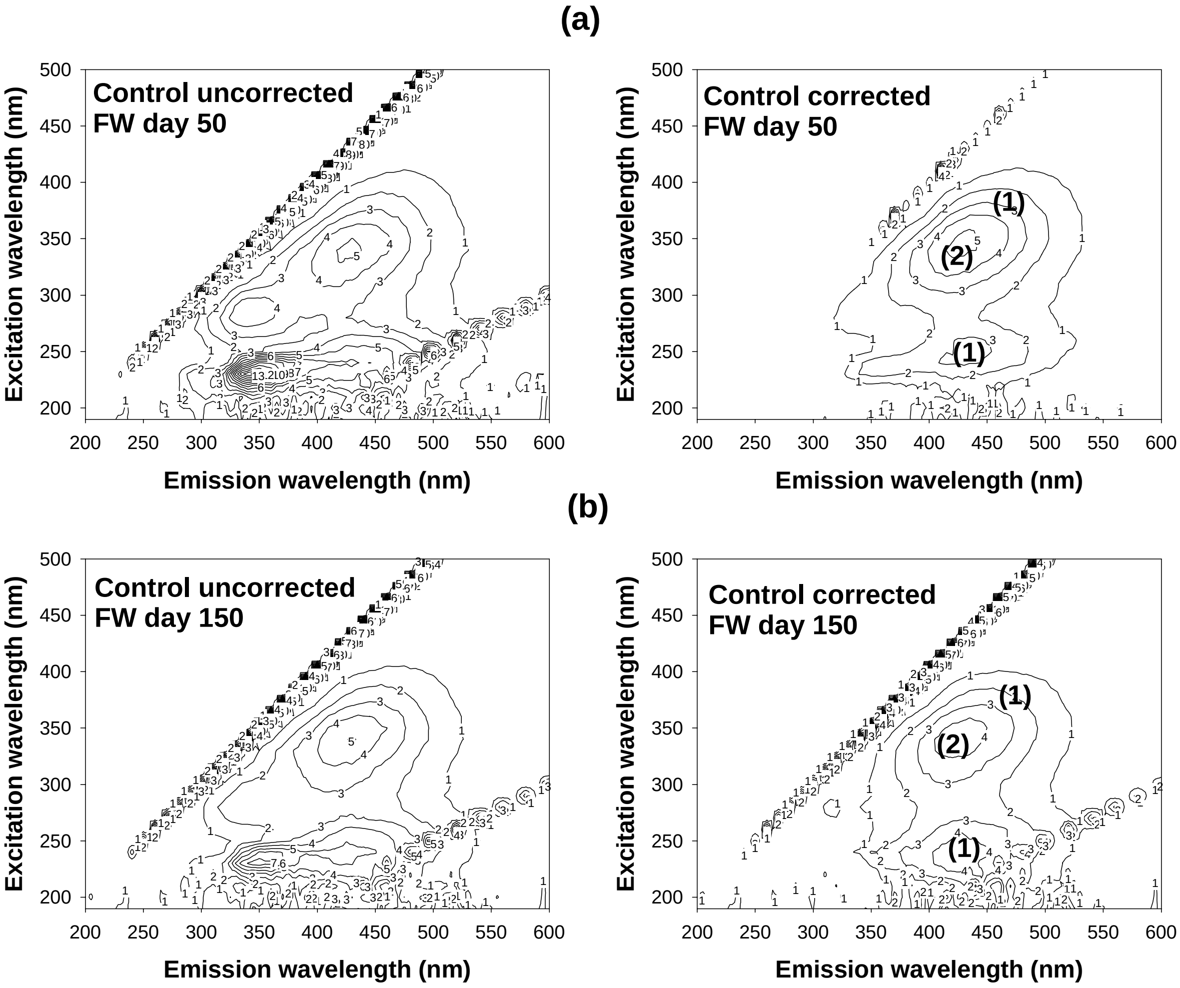


Figure 5.11 3D EEM fluorescence spectra of fresh waste (FW) leachates at day (a) 50, (b) 150 (dilution factor 25)

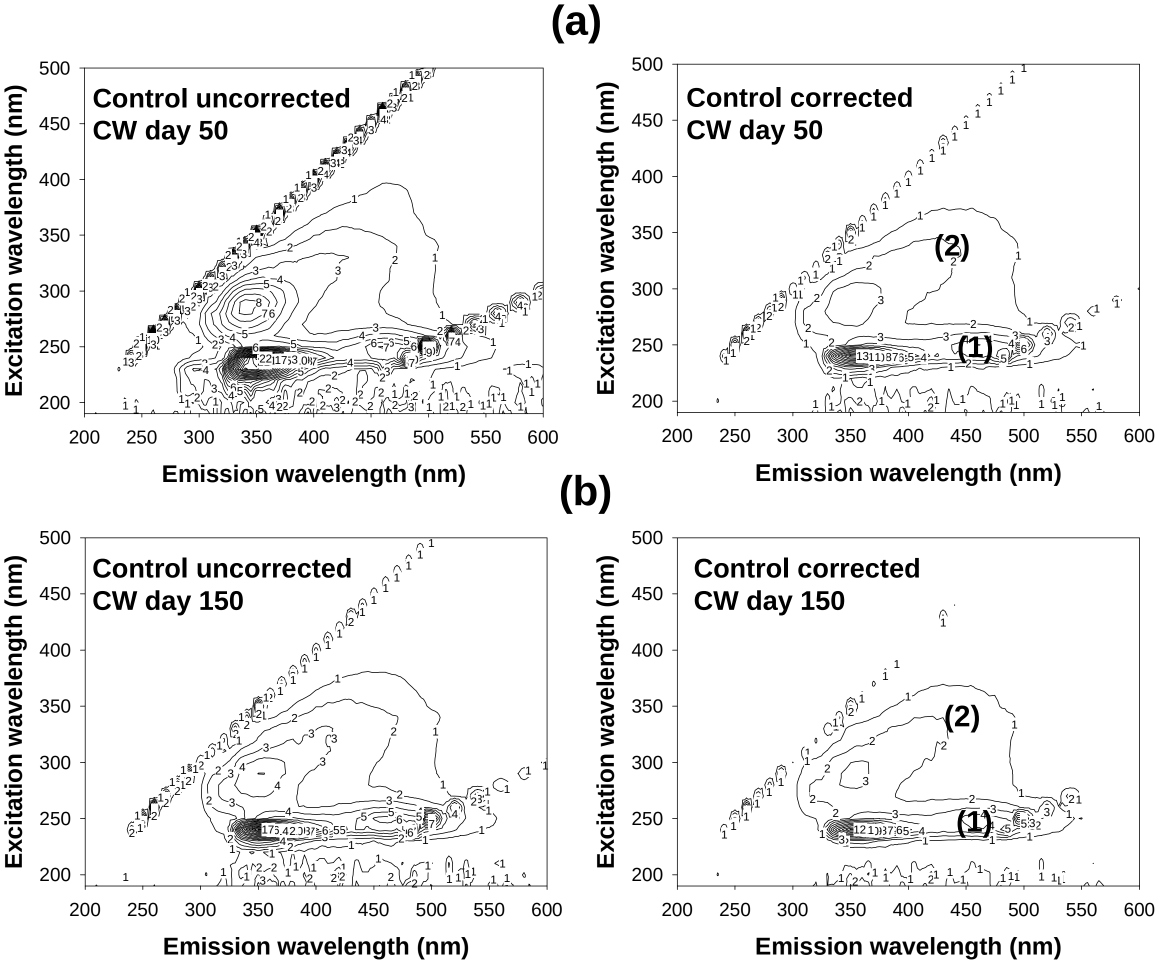


Figure 5.12 3D EEM fluorescence spectra of composted waste (CW) leachates at day (a) 50 and (b) 150 (dilution factor 50)

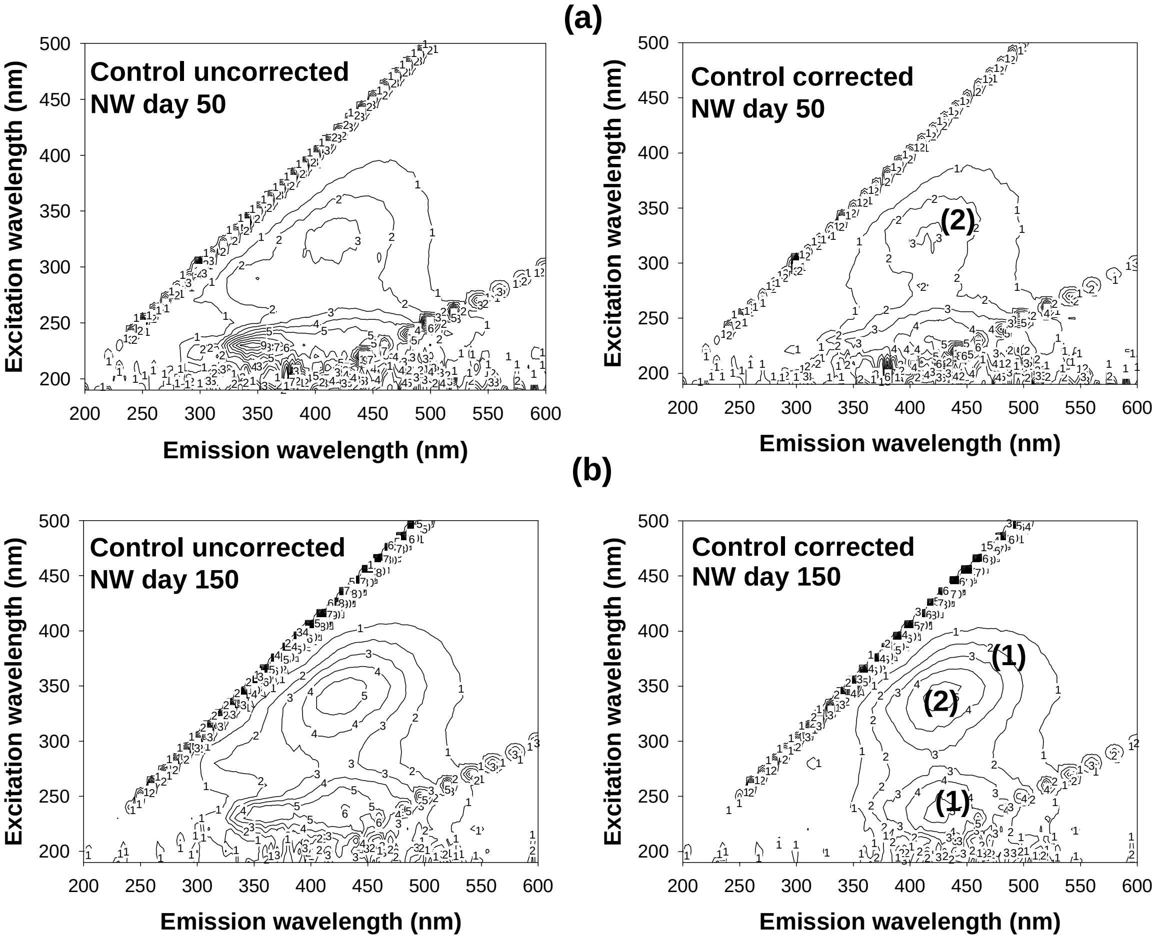


Figure 5.13 3D EEM fluorescence spectra of newspaper waste (NW) leachates at day (a) 50 and (b) 150 (dilution factor 40)

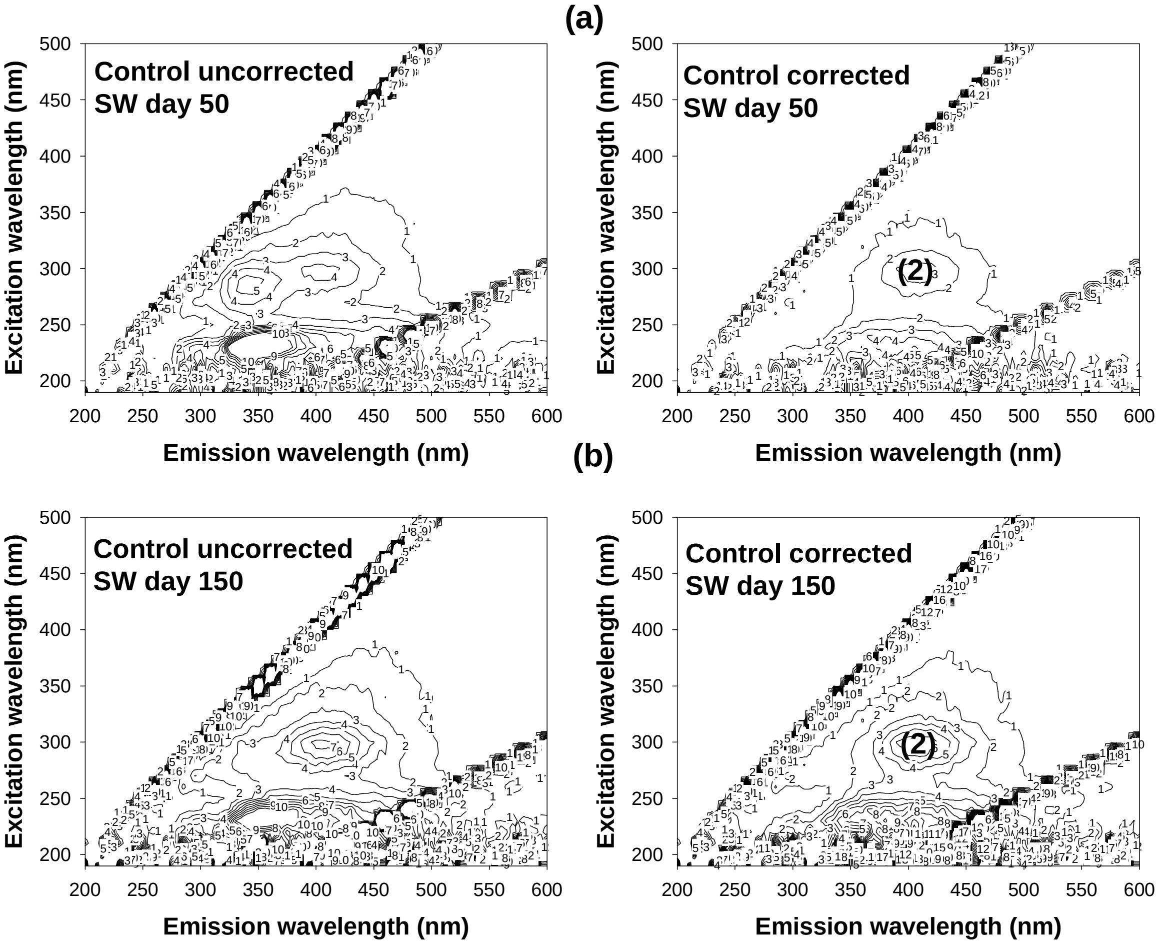


Figure 5.14 3D EEM fluorescence spectra of synthetic waste (SW) leachates at day (a) 50 and (b) 150 (dilution factor 10)

Table 5.4 shows that the intensity of H-L compounds gradually increased from day 70 to the end of the biodegradation for all leachates while a variation in the intensity of F-L compounds was observed. The changes in the intensities of H-L and F-L compounds indicated the change in concentration of these compounds during anaerobic biodegradation of the wastes. It is worth noting that the formation of H-L and F-L compounds followed the trend reported by Artiola-Fortuny and Fullar (1982) that initially leachates contain high levels of F-L compounds and as time progresses, the fractions of H-L compounds increase and the fractions of F-L compounds may decrease or remain constant depending on the biodegradation processes. As discussed before, H-L and F-L compounds are the key components of the recalcitrant organic compounds. Therefore, it can be said that this increase of fluorescence intensity of H-L and F-L compounds was an indication of the increase in the proportion of the recalcitrant organic compounds in leachates during the anaerobic biodegradation. The increase of recalcitrant organic compounds in leachates during the anaerobic biodegradation could be attributed to the results of the relatively less degradable lignin and the generation of recalcitrant products both from cellulose degradation and possibly microbial synthesis. The other reason could be the release of less degradable material from the wastes as a result of breakdown of the waste and the degradation of the readily degradable portion. A possible degradation pathway of the waste components that leads to the generation of recalcitrant organics is presented in figure 5.15. Table 5.4 also shows that the fluorescence intensities of H-L and F-L compounds in composted waste and newspaper waste leachates are significantly higher than in fresh waste and synthetic waste leachates. This indicates that composted waste and newspaper waste leachates contained relatively higher concentrations of recalcitrant organic compounds than fresh waste and synthetic waste leachates. This could be attributed to the fact that composting significantly degraded readily biodegradable organic carbon in composted waste and hence a subsequent anaerobic biodegradation of the remaining organic matter produced more biologically resistant compounds. For newspaper waste leachates, high percentage cellulose and lignin could contribute to the generation of biologically resistant compounds. The gas production trend (figure 5.1) also showed that about 94% and 87% of the total gas was produced within 70 days for composted waste and newspaper waste respectively. This implies that most of the biologically available carbon was utilised within 70 days with the remaining organic carbon being relatively resistant to biodegradation. All of these factors might play an important role in the formation of higher proportions of recalcitrant organic compounds in composted waste and newspaper waste leachates and hence high intensities of H-L and F-L compounds were observed in these leachates. To understand better the relative proportions of H-L and F-L compounds in the dissolved organic compounds in leachates, the intensities of H-L and F-L compounds were plotted against the DOC for every leachate sample (from day 50 onwards) in figure 5.16 (a-d). As can be seen from figure 5.16 (a-d), newspaper waste leachates gave the best relation between the H-L and F-L fluorescence intensity and DOC with R^2 = 0.94 and 0.89 respectively. The strongest relationship observed in newspaper waste leachates indicated that significant proportions of H- L and F- L compounds dominated the remaining DOC from day 50 onwards. In contrast, the weakest relation observed in the fresh waste leachates (H- L, R^2 = 0.32 and F- L, R^2 = 0.27) indicating that H- L and F- L compounds made up a relatively small component of the remaining DOC and significant amounts of biodegradable organic compounds were still present. Composted waste leachate also showed good relationship between these two parameters (H- L, R^2 = 0.90 and F- L, R^2 = 0.60). These results were indicative of the significant amount of recalcitrant organic compounds in newspaper waste and composted waste leachates as well. It was also observed from figure 5.16 (a-d) that for newspaper waste leachates, both H-L and F-L compounds were the significant portions of remaining DOC whereas for fresh waste and composted waste leachates, H-L compounds dominated the DOC.

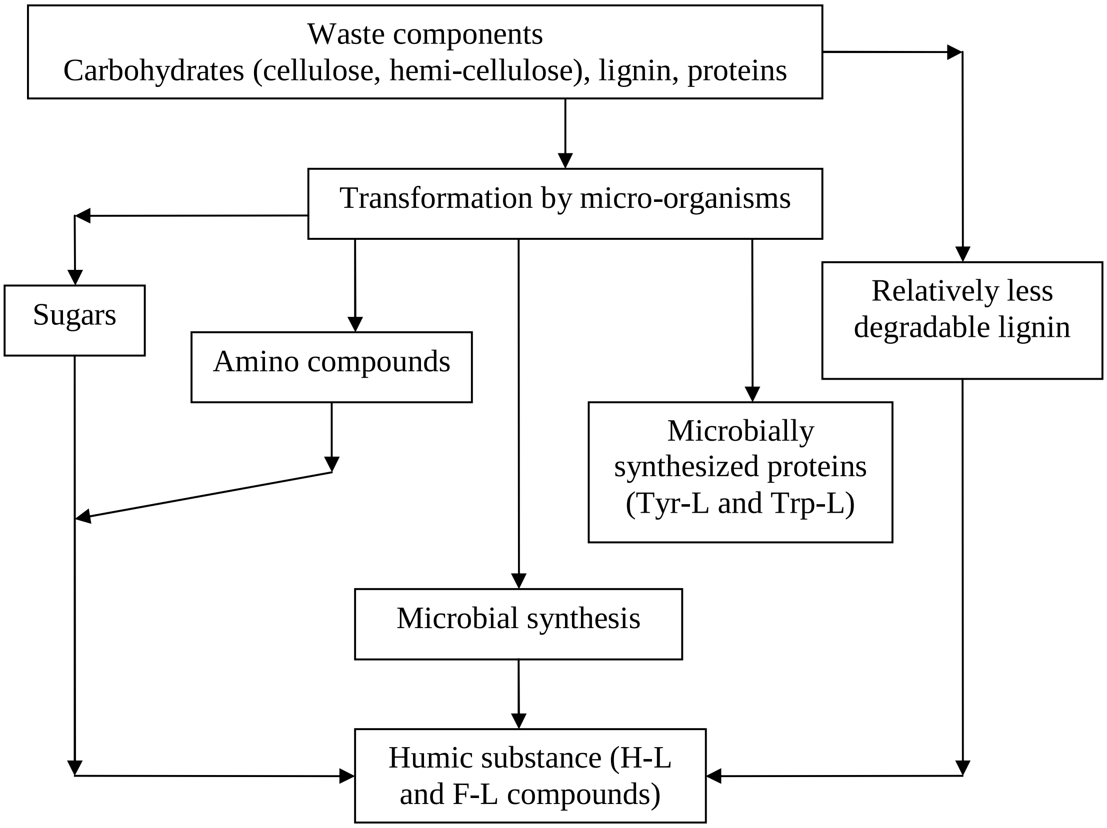
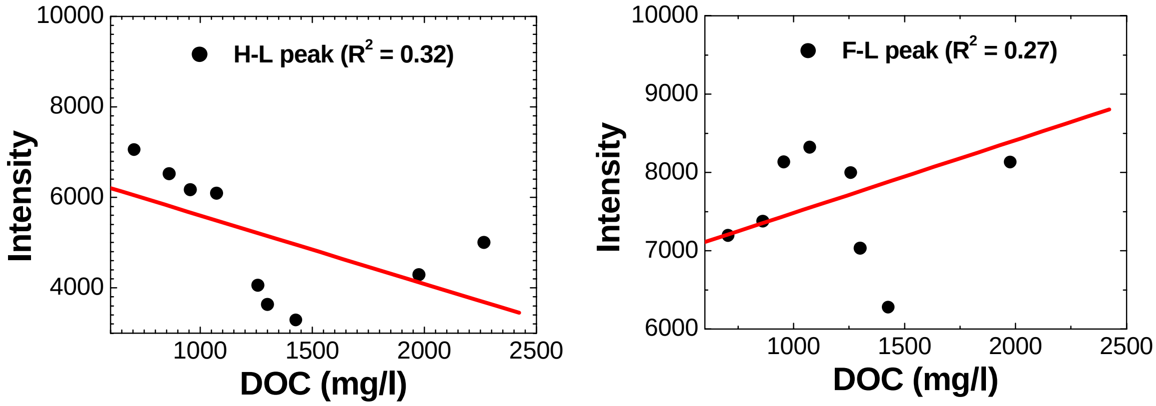


Figure 5.15 A possible degradation pathway of waste organic matter into the recalcitrant organics in leachates

(a)
(Fresh waste)



(b)
(Composted waste)

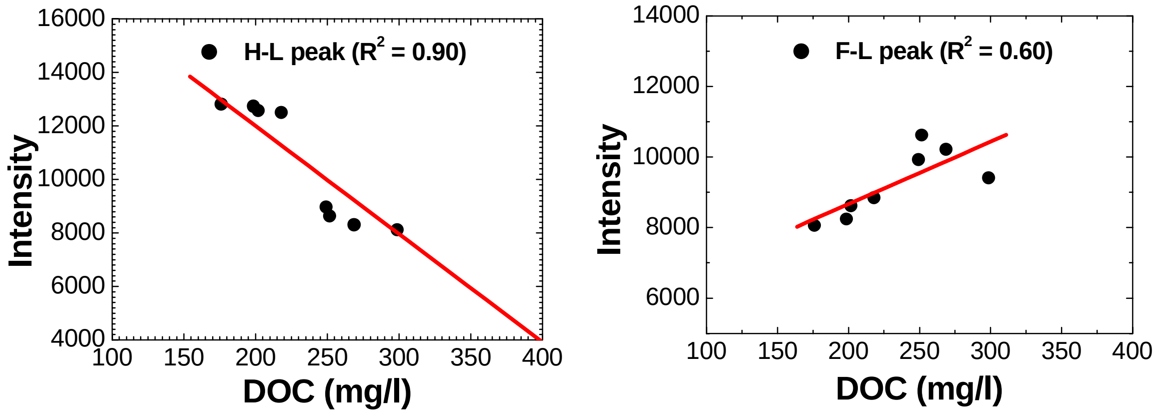
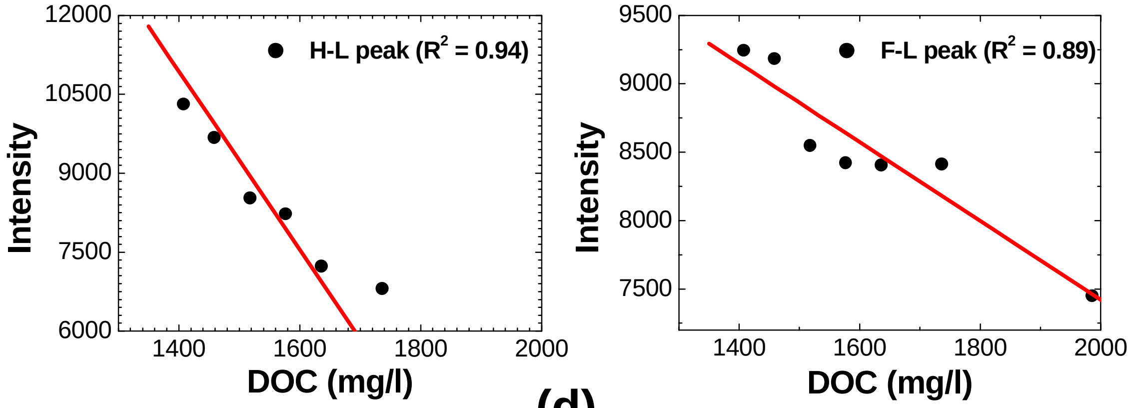


Figure 5.16 Comparisons of fluorescence intensities with DOC for (a) fresh waste leachates; H-L: y = 7109 – 1.52x; F-L: y = 6554 + 0.93x and (b) composted waste leachates; H-L: y = 22355 – 51x; F-L: y = 5125 + 18x

(c)
(Newspaper waste)



(d)
(Synthetic waste)

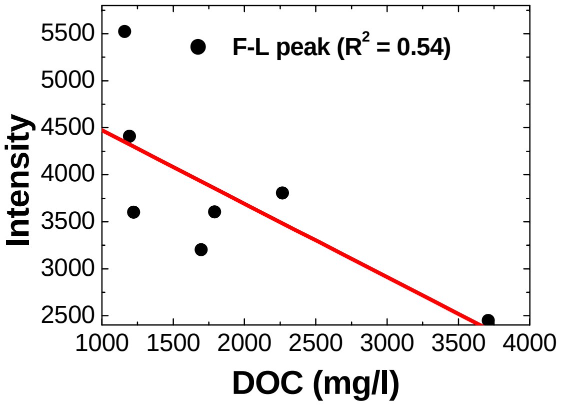


Figure 5.16 (contd.) Comparisons of fluorescence intensities with DOC for (c) newspaper waste leachates; H-L: y = 34711 – 17x; F-L: y = 13180 – 2.9x and (d) synthetic waste leachates; F-L: y = 5254 - 0.8x

Regarding the protein-like compounds, the intensities of Tyr-L and Trp-L compounds in general followed a decreasing trend over the whole period of anaerobic biodegradation for fresh waste, composted waste and newspaper waste leachates and an increasing trend for synthetic waste leachates. This implies the general biodegradability of the protein like compounds in fresh waste, composted waste and newspaper waste leachates. However, for fresh waste and newspaper waste leachates, protein-like compounds accumulated from day 50 to day 70, i.e. intensity of Trp-L compounds increased (Table 5.4). This increase might be explained by the release of protein from the wastes through the degradation of structures and also by the production of protein from microbial synthesis (Wu, 2002). This increase of proteins from day 50 to 70 in fresh waste leachate could also favour the intensity decrease of H-L and F-L compounds through the sorption by lipophilic extractives as explained previously. The increasing trend of Trp-L compounds observed in synthetic waste leachates might also be explained by the protein-like compounds generated by waste degradation and microbial synthesis. From Table 5.4, it was also found that the intensities of protein-like compounds for composted waste leachates were significantly higher than for the other leachates. This indicates a significant protein transfer into the composted waste leachate. However, the carbon mass balance of different wastes (section 5.4) where carbon transfer in biomass form cannot be taken into account showed that the lowest amount of utilized total carbon was transferred into the composted waste leachate. This result simply indicates that majority of the total carbon which was transferred into leachate in protein form might be transferred as bacterial biomass (Ivanova, 2007).

The ratios of H-L/F-L over time can be used as an indicator of waste biodegradation and are presented in figure 5.17 for fresh waste, composted waste and newspaper waste leachates (the ratio of H-L/F-L for synthetic waste leachates was not calculated as H-L compounds were not observed in this leachate). The ratio of H-L/F-L showed the existence of three phases of anaerobic biodegradation in the fresh waste leachates. From day 50 to day 70 (termed as phase 1) the ratios of H-L/F-L remained constant. The ratio decreased during the second phase (from day 70 to day 90) possibly because of the synthesis of F-L compounds. Thereafter in the last phase (from day 100 to the end of the test) the ratios increased by the increase of H-L compounds and decrease of F-L compounds. However, in composted waste leachates, phase 1 was absent. Contrary to the fresh waste and composted waste leachates, only the phase 3 was present in newspaper leachates as the decrease in H-L/F-L ratio was not observed in this leachates. The values of the H-L/F-L ratio at the end of the biodegradation in the leachates followed the sequence: composted waste>newspaper waste>fresh waste.

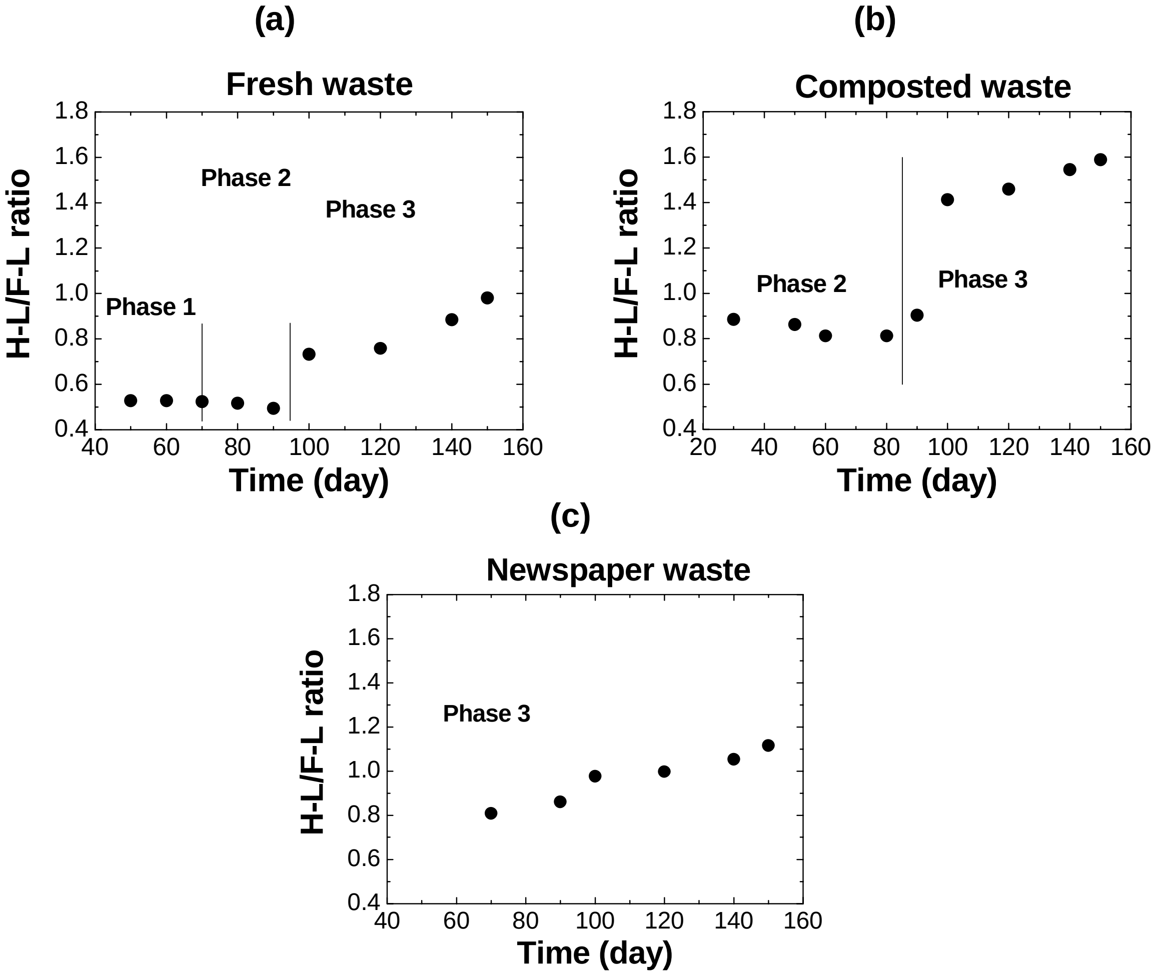


Figure 5.17 H-L/F-L ratio and phases in the fresh waste, composted waste and newspaper waste leachates

The above results suggest that fluorescence spectroscopy may be a suitable tool for investigating the recalcitrant organic compounds formed in leachates during the anaerobic biodegradation of wastes. The intensity, positioning and shift of the fluorescence peaks of different leachates may be explained by the variability in biodegradability of the waste components. This could be related to the chemical and structural changes of individual organic compounds during biodegradation.

5.5.1.3 Fluorescence analyses of humic and fulvic like materials in leachates in emission scanning mode

In this section the structure of various compounds in leachates under anaerobic biodegradation of waste is considered. As discussed in Chapter 4, fluorescence spectra in emission scanning modes provide information about the aromatic structure of compounds. Figure 5.18 (a-d) presents the fluorescence emission characteristics at specified excitation wavelengths for leachates taken from four different wastes. The leachates from all four wastes exhibited two characteristic peaks denoted Peak I (at a shorter wavelength of 340-350 nm) and Peak II (at a longer wavelength of 430 nm). Kang et al. (2002) reported that the shorter wavelength peaks (360 nm) can be interpreted as being due mainly to the presence of simple aromatic rings in the molecule, whereas the peaks in the longer wavelength (430 nm) are due to the condensed aromatic rings and conjugation of simple aromatic rings.

Figure 5.18 (a-d) shows that the intensity of Peak II at (300-390) nm excitation was stronger than that of Peak I in all of the leachates. For composted waste leachates, Peak I at (230-260) nm excitation had the highest intensity. As compared with the EEM spectrum (figures 5.11, 5.12, 5.13 and 5.14), Peak I can be assigned as Trp-L fluorophore. Peak II at (230-260) nm excitation is located in the range of H-L and at (300-390) nm excitations in the range of F-L fluorophores. It was found that for composted waste leachates; Peak II at (230-260) nm excitation was in a higher wavelength region (450 nm) than for the other leachates. This may mean that H-L compounds in composted waste leachates contained more condensed forms of aromatic rings than the other leachates. It was also observed that for synthetic waste leachates; the Peak II at (270-340) nm excitation was in the region around 400 nm, indicating that F-L compounds in synthetic waste leachates contained less condensed forms of aromatic rings than the other leachates.

From figure 5.18 (a-d), the intensity of Peak II increased during 150 days of anaerobic biodegradation for all leachates. This suggests that condensation of the aromatic structure of H-L and F-L molecules increased over time.

Figure 5.18 (a-d) also shows that the ratio of intensities of the peaks of longer wavelength to the shorter wavelengths for newspaper waste leachates was higher than for fresh waste and synthetic waste leachates. This implies that newspaper waste leachates contained more condensed forms of aromatic structures than fresh waste and synthetic waste leachates. In addition, it appears that composted waste leachates contained more condensed forms of aromatic H-L compounds than the other leachates. These fluorescence spectroscopic results led to the conclusion that composted waste and newspaper waste would contribute to leachates with higher concentrations and more condensed forms of recalcitrant H-L and F-L compounds than fresh waste and synthetic waste.

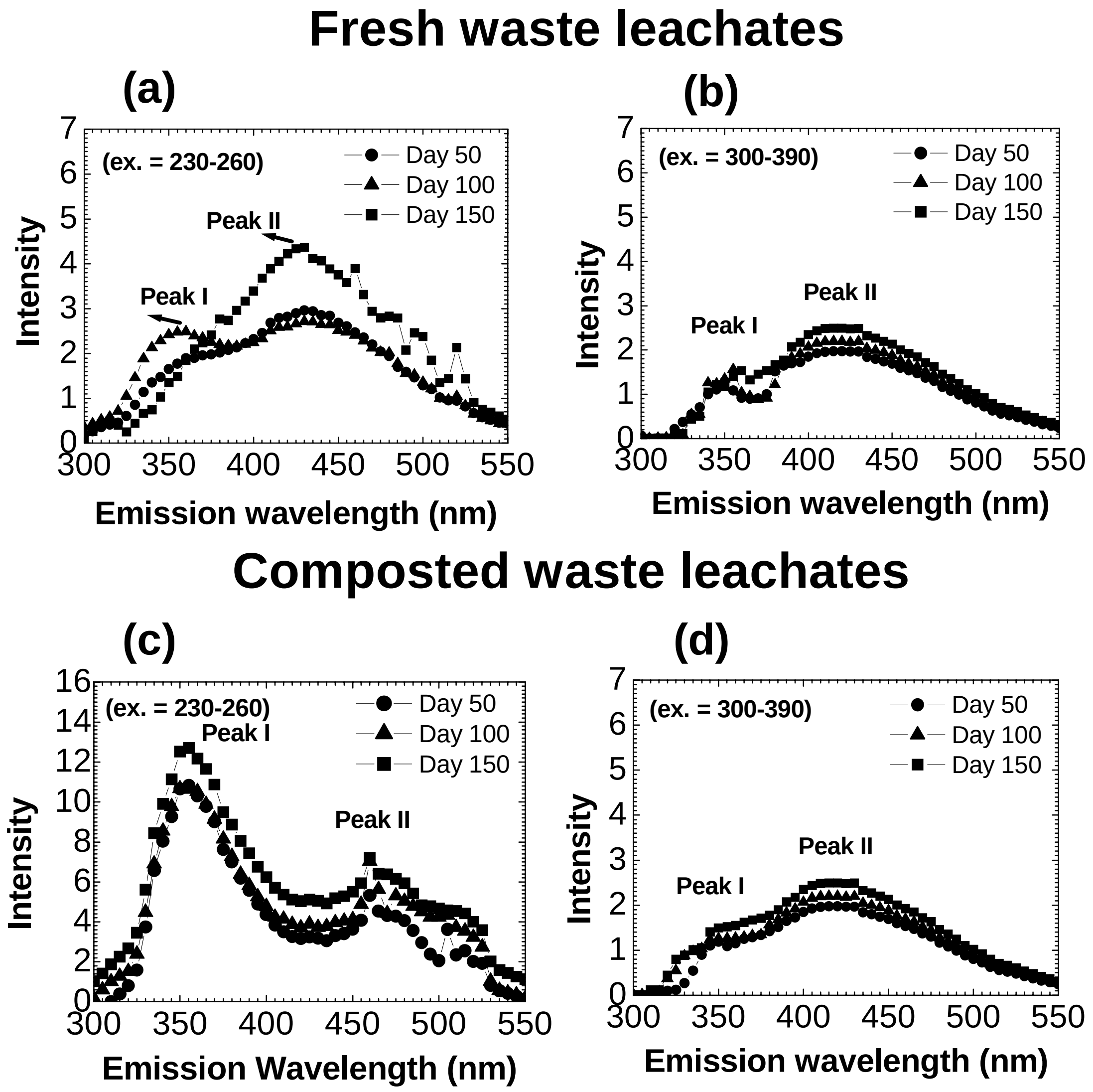


Figure 5.18 Fluorescence spectra of fresh waste (a, b) and composted waste (c, d) leachate samples in emission scanning mode at excitation wavelengths 230-260 nm and 300-390 nm (dilution factor 25 and 50 for fresh waste and composted waste respectively)

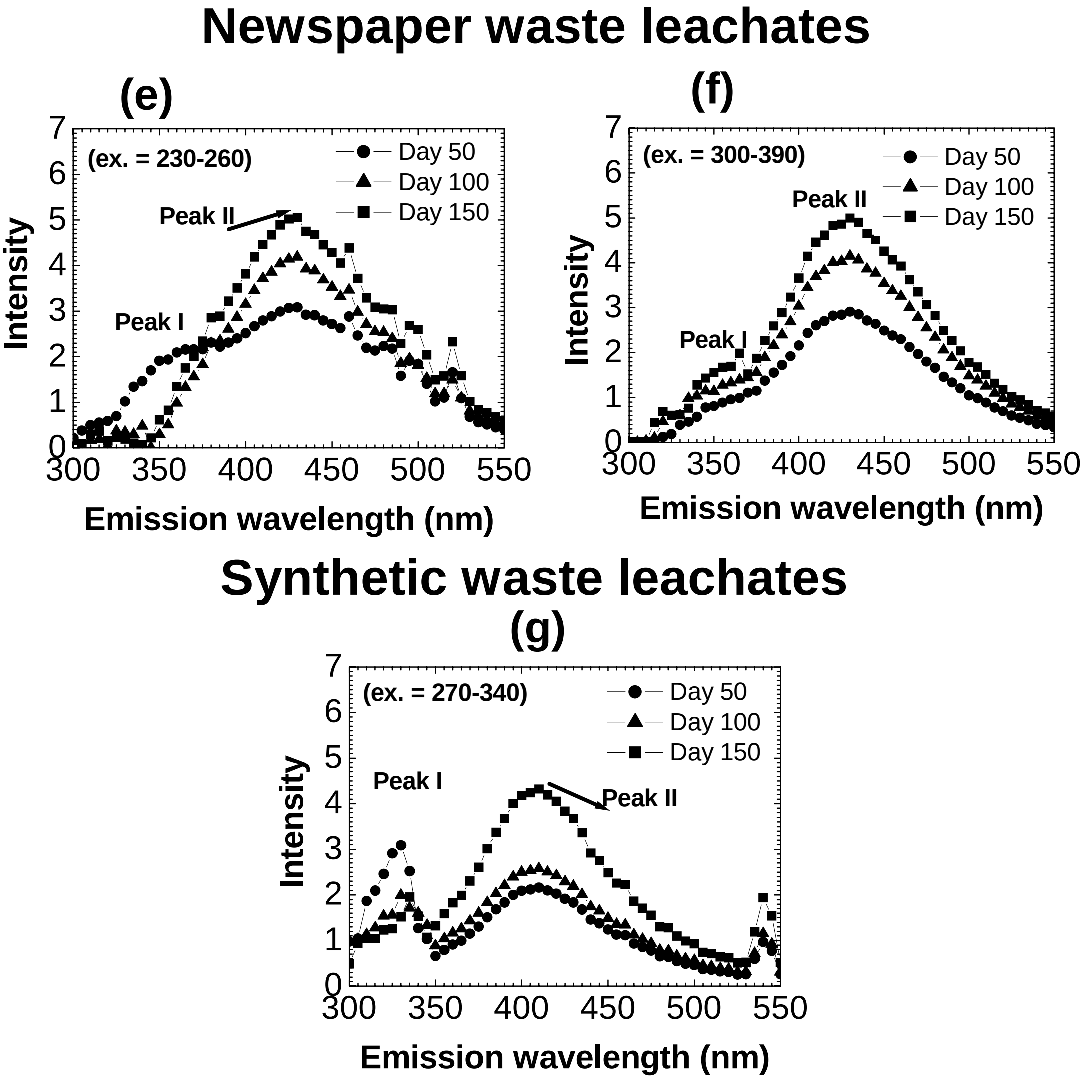


Figure 5.18 (contd.) Fluorescence spectra of newspaper waste (e, f) and synthetic waste (g) leachate samples in emission scanning mode at excitation wavelengths 230-260 nm and 300-390 nm (for synthetic waste, excitation wavelength was 270-340 nm) (dilution factor 40 and 10 for newspaper waste and synthetic waste respectively)

5.5.2 UV spectroscopic results

As discussed in Chapter 2, the specific UV absorbance (SUVA) can be used to study the aromaticity of leachates. Figure 5.19 shows the SUVA calculated for fresh waste, composted waste, newspaper waste and synthetic waste leachates with time during biodegradation. It was found that the SUVA of these leachates increased with time, indicating the concentration of aromatic molecules increased with the waste biodegradation. This result is in agreement with the previous findings reported by Kang et al. (2002) and Fan et al. (2006). At the end of biodegradation, the SUVA values for fresh waste and synthetic waste leachates were approximately 0.065 and 0.06 respectively. The SUVA of newspaper waste leachates was about twice this value, whereas the SUVA of composted waste leachates was 30 times as great implying that the molecular characteristics of the composted waste and newspaper waste leachates were more aromatic in nature.

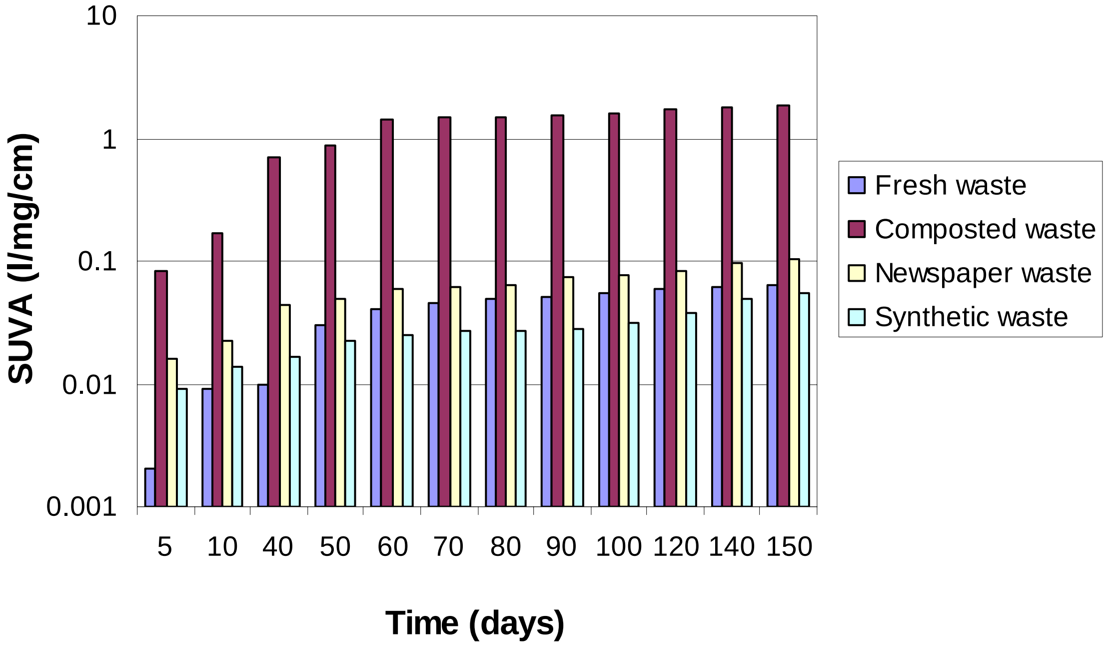


Figure 5.19 The SUVA for fresh waste, composted waste, newspaper waste and synthetic waste leachates (dilution factor 25, 50, 40 and 10 for fresh waste, composted waste, newspaper waste and synthetic waste respectively)

The ratio of absorbance at 465 nm and 665 nm (E_4/E_6) can provide an indication of the molecular size of leachates (Kang et al., 2002). Figure 5.20 shows the E_4/E_6 ratio of fresh waste, composted waste, newspaper waste and synthetic waste leachates which decreased over time. It is known that the E_4/E_6 ratio is inversely proportional to molecular weight, which implies that the E_4/E_6 ratio decreases with increasing molecular weight. Therefore, the decrease of the E_4/E_6 ratio indicates that the concentration of high molecular weight molecules increased with waste biodegradation. This agrees with the results reported by Kang et al. (2002).

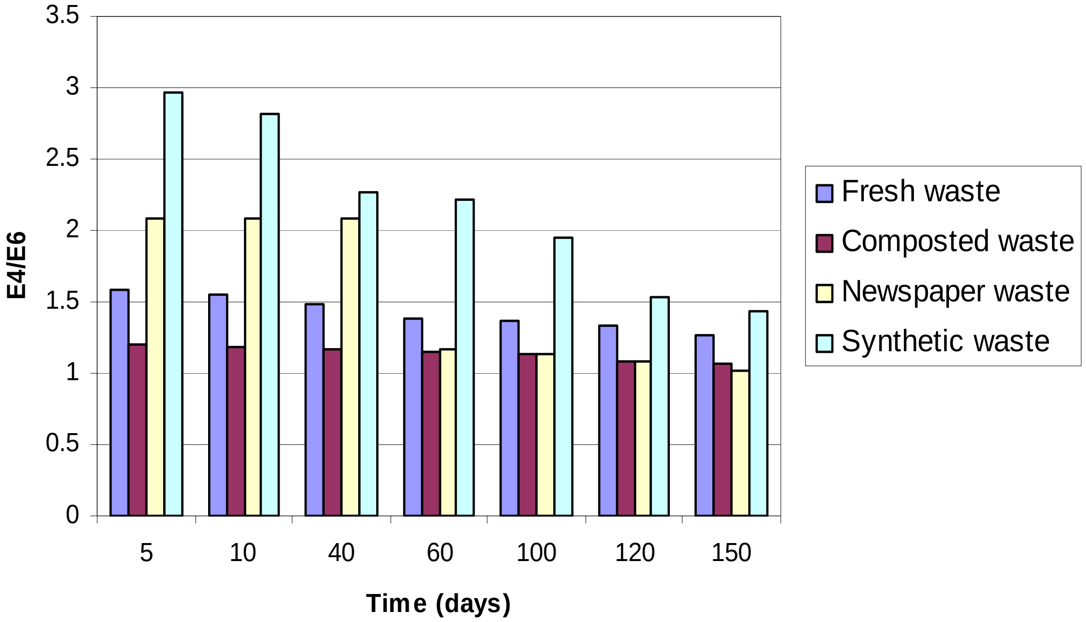


Figure 5.20 E_4/E_6 ratio for fresh waste, composted waste, newspaper waste and synthetic waste leachates (dilution factor 25, 50, 40 and 10 for fresh waste, composted waste, newspaper waste and synthetic waste respectively)

These above results can be correlated with the general trend observed in fluorescence spectroscopic results. They suggest that waste biodegradation increased aromaticity and the molecular weight of organic compounds in leachates. Composted waste and newspaper waste leachates had the higher fluorescence intensities indicating a more condensed form of aromatic structure of H-L and F-L compounds than fresh waste and synthetic waste leachates. As aromaticity is one of the main characteristics of recalcitrant H-L and F-L compounds, the UV and fluorescence spectroscopic results allow us to conclude that leachates produced during the anaerobic degradation of composted waste and newspaper waste may contain a higher proportion of condensed forms of aromatic organic compounds than fresh waste and our synthetic waste leachates.

5.6 Summary

In this chapter, the evolution and characteristics of recalcitrant organic compounds in leachates during anaerobic biodegradation of four types of solid waste was investigated. Biochemical Methane Potential (BMP) tests were carried out on fresh waste, composted waste, newspaper waste and synthetic waste over a period of 150 days and leachates produced from the degradation of all four wastes were characterised using conventional methods as well as by UV absorption and fluorescence spectroscopy. The increase of the fluorescence intensities related to humic (H-L) and fulvic (F-L) compounds in the leachates formed during anaerobic biodegradation was considered to be indicative of the increased proportion of recalcitrant organic compounds. The recalcitrant organic compounds (H-L and F-L compounds) in all four leachates were influenced by the nature of the wastes and also the anaerobic biodegradability of the component solid materials. It was estimated that the total carbon released from solid waste which was transferred to leachate and gas was low in composted waste because of the amount of biodegradable organic compounds that had been removed during composting. Subsequent anaerobic biodegradation of the remaining organic compounds produced more biologically resistant compounds. Additionally, the relatively less resistant cellulose and high lignin present in newspaper waste contributed to an increase in the proportion of recalcitrant organic compounds in the leachate. As expected, trends in biochemical composition of waste and gas production indicated that fresh waste and synthetic waste had a higher biodegradation potential than composted waste and newspaper waste.

Fluorescence and UV spectroscopic results led us to conclude that composted waste and newspaper waste would contribute to the generation of higher concentrations of more condensed aromatic structures of recalcitrant H-L and F-L compounds than fresh waste and synthetic waste. These results indicate that in a landfill system, composted waste and newspaper waste would generally result in a higher concentration of recalcitrant organic compounds than fresh waste and synthetic waste.

Chapter 6

Conclusions and future work

6.1 Conclusions

This research investigated the characteristics of recalcitrant organic compounds in landfill leachates and also studied the possible use of UV absorption and fluorescence spectroscopy for rapid characterisation of these compounds in leachates. These investigations comprised both studies of leachates collected from two UK landfill sites (Pitsea and Rainham) and of leachates generated from laboratory scale anaerobic biodegradation of solid waste components. This fulfils the research objective of understanding the nature of the recalcitrant organic compounds in leachates in course of a biological treatment processes, comparing the biodegradation associated evolution of the recalcitrant organic compounds in two UK landfill sites and assessing the influence of individual waste types on the recalcitrant organic compounds. The investigation of the recalcitrant organics was carried out by established methods of COD and DOC measurements, and by the potential new techniques, UV absorption and fluorescence spectroscopy. The UV and fluorescence spectroscopy provided rapid ways of monitoring some constituent compounds of leachates and the biodegradation of leachates were studied by estimating the eventual removal of the measurable variables in course of the treatment. UV spectroscopy was used to characterize the aromatic compounds whereas the fluorescence spectroscopy was used for fingerprinting different organic compounds such as humic, fulvic, proteins in leachates and their biodegradation. The biodegradability of different leachates thus indicated by the UV and fluorescence spectroscopy was compared with the results of conventional methods to assess the validity of the new approaches. This investigation satisfied the research objective of the basic feasibility study on the application of UV absorption and fluorescence spectroscopy for a rapid and an economical characterisation of the organic compounds in landfill leachates and also provided insight into the recalcitrant organic compounds.

Leachate samples were collected from two UK MSW landfills, Pitsea and Rainham in Essex and a laboratory scale aerobic biological treatment was carried out in glass reactors over a period of 30 days with an air supply arrangement. The conventional methods of characterisation demonstrated the expected gradual decrease of total/dissolved chemical oxygen demand (COD) and total/dissolved organic carbon (DOC) in all leachates indicating the reduction of organic compounds by the aerobic biological treatment. The observed COD and DOC values of Pitsea (P4), Rainham (P2) and Pitsea (LTP) leachates were considerably higher than Rainham (LTP), Rainham (FE) and Rainham (LTP Haz) leachates indicating that Pitsea (P4), Rainham (P2) and Pitsea (LTP) leachates contained comparably higher amounts of organic compounds than Rainham (LTP), Rainham (FE) and Rainham (LTP Haz) leachates. In addition, no discernible difference was observed between total and dissolved carbon contents of different leachates which indicated that the organic compounds in these leachates were mostly in dissolved form. The biodegradability of different leachates was estimated from the remaining percentages of DOC and COD in course of the treatment. The results of %DOC reduction trend showed that the Rainham (LTP) and Rainham (FE) leachates which had lower concentrations of organic compounds were more easily biodegradable leachates whereas the Pitsea (LTP), Pitsea (P4) and Rainham (P2) leachates which had higher concentration of organic compounds were not so easily biodegradable.

A study of constituent organic compounds during biodegradation of leachates using UV absorption and fluorescence spectroscopy provided for a rapid investigation and insight into the recalcitrant organic compounds in leachates. It was found that leachates with high UV_{254} absorbance values exhibited a low percentage reduction of UV_{254} absorbance and DOC in course of the treatment. This suggested that leachates containing high aromatic organic compounds usually result in low percentage removal of DOC in the course of a treatment process. UV absorption allowed the study of the effect of biodegrdation on different leachates through the reduction of aromatic compounds in course of the treatment. The study also showed that Rainham (LTP) and Rainham (FE) leachates were more easily biodegradable than the Pitsea (LTP), Pitsea (P4) and Rainham (P2) leachates which were in agreement with results estimated using conventional methods. The good agreement in the degradation trends observed in different leachates by UV_{254} absorption and DOC measurements probably indicated that organic compounds contributing DOC in these leachates were mostly aromatic in nature. Fluorescence spectroscopy was used to study the biodegradation of organic compounds such as humic, fulvic, proteins in leachates. This study in general showed a lower reduction of the fluorescence intensities of the humic-like (H-L) and fulvic-like (F-L) compounds in comparison to the protein-like compounds for all leachates in course of the biodegradation. This indicated that humic-like (H-L) and fulvic-like (F-L) compounds were the key components of recalcitrant organic compounds. It was also found that the reduction of the fluorescence intensities of the humic-like (H-L) and fulvic-like (F-L) compounds in Rainham (LTP) and Rainham (FE) lechates were around 60% after 30 days of aeration whereas this value for Pitsea (LTP), Pitsea (P4) and Rainham (P2) leachates were around 4 to 30%. This result indicated the biodegradation potential of different leachates which were in agreement with UV spectroscopy and conventional DOC measurement results. Although %COD removal results were not an exact replica of %DOC removal and spectroscopic results, in general this was also in fair agreement, as %COD removal was also higher for Rainham (LTP) leachate than for Pitsea (P4) leachate. Thus the general agreement observed between the spectroscopic method and conventional methods in assessing biodegradability of different leachates simply pointed to the fact that a rapid characterisation of the organic components of landfill leachate that were more resistant to aerobic degradation using spectroscopic method was reliable.

To understand the influence of different types of waste on the generation of recalcitrant organic compounds in leachates, an anaerobic biodegradation experiment was carried out for four types of wastes, i.e., fresh waste, composted waste, newspaper waste and synthetic waste in the BMP test reactors and leachates generated during biodegradation processes were characterized. The biodegradation processes took place over a period of 150 days at approximately 30^{o}C. General waste biodegradation was first studied through different measurements. The loss of cellulose, hemi-cellulose and lignin in the waste samples due to biodegradation was estimated by the measurements of Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Digestible Lignin (ADL). As expected it was found that composted waste contained the lowest percentages of cellulose and hemi-cellulose of all of the wastes. The percentage of lignin in composted waste and newspaper waste was found to be higher than in fresh waste and synthetic waste. The estimated cellulose and hemi-cellulose degradation after 150 days of anaerobic biodegradation were 46%, 32%, 54%, 41% and 10.5%, 8.3%, 18.7% and 8% for fresh waste, composted waste, newspaper waste and synthetic waste respectively. Thus cellulose showed a better degradation trend than hemi-cellulose in all of these wastes. The lignin fraction after 150 days of biodegradation was barely affected in all these waste samples. This indicated the low degradation of lignin and relatively high degradation of cellulose and hemi-cellulose over time and thereby a mild increase in the percentage of lignin after 150 days of biodegradation. Considering cellulose and hemi-cellulose degradation, composted waste was found to be least biodegradable among the four wastes under consideration. The low biodegradation of composted waste was as expected and could be explained by the fact that most of the readily biodegradable cellulose and hemi-cellulose in the original waste would already have been degraded during composting. Lignin was known to significantly reduce the biodegradation primarily through sheathing of cellulose and hemi-cellulose. The (Cellulose + Hemi-cellulose) to Lignin ratio, (C+H)/L was also used to asses the extent of biodegradation of different wastes. After 150 days of anaerobic biodegradation, the (C+H)/L values of fresh waste, newspaper waste and synthetic waste fell from 2.3, 2.81 and 3.5 to 1.23, 1.2 and 1.58 respectively, while the (C+H)/L ratio of the composted waste fell from 0.9 to 0.56. This result again indicated the low biodegradation potential of composted waste. The carbon mass balance suggested that 8.5% (fresh waste), 7.0% (composted waste), 10.5% (newspaper waste) and 15% (synthetic waste) of utilized total carbon was transferred into the leachate and 39% (fresh waste), 15% (composted waste), 22% (newspaper waste) and 48% (synthetic waste) of utilized total carbon was transferred into the biogas by the end of 150-day monitoring period. Thus mass balance analysis in general allowed us to rate the observed biodegradation in these wastes as synthetic waste > fresh waste > newspaper waste > composted waste. It was interesting to note that the lower mass transfer of newspaper waste into gas (22%) in comparison to the synthetic waste (48%) and fresh waste (39%) was also reflected in the cumulative gas production after 150 days. The total produced gas was found to be highest for fresh waste and synthetic waste and lowest for composted waste. The newspaper waste exhibited an intermediate gas production.

Leachates generated in the BMP test reactors were also taken periodically and investigations were performed to determine the composition of the leachates generated during different stages of waste biodegradation. Conventional method of characterisation showed that TOC values increased from initial values of 1308 mg/l to 4625 mg/l after 35 days, 1813 mg/l after 5 days, 3888 mg/l after 25 days and 5468 mg/l after 22 days and then decreased gradually to levels of 705 mg/l, 176 mg/l, 1408 mg/l and 1160 mg/l for fresh waste, composted waste, newspaper waste and synthetic waste respectively by the end of the test. A similar trend was observed for dissolved organic carbon (DOC) and total chemical oxygen demand (TCOD) values of the leachate samples in course of biodegradation. This initial increase was explained by the transfer of the solid organic matter into leachates from waste although biodegradation of organic materials in leachates was simultaneously active. Once waste biodegradation reached certain levels, biodegradation of leachates dominated thereby reducing DOC, COD values. However, it was observed that total organic carbon (TOC), DOC, chemical oxygen demand (TCOD and DCOD) values for fresh waste, newspaper waste and synthetic waste leachates were significantly higher than for composted waste leachates. In addition, the TOC value in composted waste leachate reached its peak value within only 5 days. These results indicated that, as expected there was a lower organic contents in composted waste leachates in comparison to other leachates. It was also suggested that further removal of organic compounds might be possible through biodegradation in fresh waste, newspaper waste and synthetic waste leachates. It is worth mentioning that unlike the landfill samples the biodegradability of these laboratory scale waste generated leachates was not estimated from the remaining percentages of DOC and COD due the possibility of continuous mass transfer from the wastes.

Fluorescence spectroscopy provided an excellent way to study recalcitrant materials in this case by estimating humic-like (H-L) and fulvic-like (F-L) compounds which were known to be the key components of the recalcitrant organic compounds. It was found that the fluorescence intensities of humic-like (H-L) and fulvic-like (F-L) compounds in composted waste and newspaper waste leachates were significantly higher than in fresh waste and synthetic waste leachates. As expected, this indicated that composted waste and newspaper waste leachates contained relatively higher concentrations of recalcitrant organic compounds than fresh waste and synthetic waste leachates. The presence of high proportions of recalcitrant organic compounds in composted waste leachate was explained by the fact that composting significantly degraded readily biodegradable organic carbon and hence a subsequent anaerobic biodegradation of the remaining organic matter produced more biologically resistant compounds. For newspaper waste leachates, a high percentage of cellulose and lignin could contribute to the generation of biologically resistant compounds. In addition, a low mass transfer of newspaper waste into gas would also suggest recalcitrant material formation in leachates through subsequent biodegradation. Fluorescence spectroscopy also revealed that humic-like (H-L) fluorophores of composted waste leachates were in the long emission wavelengths thereby indicated that H-L fluorophores of this leachate was more aromatic and higher molecular weight than those of the other leachates. The biodegradability of the laboratory scale waste generated leachates was studied as well by estimating the evolution of aromatic compounds using the specific UV absorbance (SUVA). At the end of biodegradation, the SUVA values for fresh waste and synthetic waste leachates were approximately 0.065 and 0.06 respectively. The SUVA of newspaper waste leachates was about twice this value, whereas the SUVA of composted waste leachates was 30 times as great implying that the molecular characteristics of the composted waste and newspaper waste leachates were more aromatic in nature. The ratio of absorbance at 465 nm and 665 nm (E_4/E_6) was also used to provide an indication of the molecular size of these leachates. It was known that the E_4/E_6 ratio was inversely proportional to molecular weight, which implied that the E_4/E_6 ratio decreased with increased molecular weight. After 150 days of biodegradation the lowest values of E_4/E_6 ratio were observed for composted and newspaper waste leachates thereby indicating the molecular complexity and the recalcitrant nature of the leachates generated from the biodegradation of composted and newspaper wastes.

This aforementioned research supported the application of UV and fluorescence spectroscopy for a rapid and on-site monitoring of landfill leachates that might help scientist and engineers to assess leachate quality, identify some groups of organic compounds and to optimise leachate treatment processes. The analyses performed on Pitsea and Rainham leachates were a promising step towards developing a database of representative information for leachates from different UK landfills. While a gradual reduction of organic compounds in course of a biodegradation is generally expected, this research made a systematic and comparative analysis on the degradation of organic compounds through conventional methods, aromaticity of organic compounds through UV absorption and on the evolution, structural strengths of some groups of organic compounds through fluorescence spectroscopy for leachates collected from different phases of Pitsea and Rainham sites. This analysis revealed that organic compounds in Pitsea (LTP), Pitsea (P4), Rainham (P2) leachates were more recalcitrant in nature in comparison to Rainham (LTP), Rainham (FE) leachates and hence, it can be unambiguously decided that Pitsea (LTP), Pitsea (P4), Rainham (P2) would require more rigorous treatment than Rainham (LTP), Rainham (FE) leachates to meet effluent standard. This research also provided an understanding of the evolution of recalcitrant materials from different wastes and it can be conclude that deposited wastes enriched with composted and newspaper waste would initially tend to produce leachates with higher concentrations and more condensed aromatic structures of recalcitrant organic compounds than fresh waste in a landfill system until all readily degradable carbon was utilised.

6.2 Future work

This study shows a way of fingerprinting and estimating the constituents of recalcitrant organic compounds using UV and fluorescence spectroscopy. An extensive application of these techniques to study the impact of various pre-treatment processes that would help to reduce the recalcitrant organic compounds in leachate could be done. This would give useful information about the necessary treatments in reducing some of the components of recalcitrant substances and also would help to devise appropriate treatment processes to be applied at the landfill sites.

While the nature of recalcitrant organic compounds in landfill leachates could indicate the necessary treatments, an interesting research topic would also be the characterisation of leachates samples collected from different landfills of varying ages, composition of wastes and sizes to develop a database of representative information of leachates' chemical composition of different UK landfills. In particular the research should target the investigation of chemical and structural composition of humic, fulvic or any other compounds that could potentially be detected by fluorescence spectroscopy to forecast the required treatment for any particular landfill site.

In addition to the above mentioned research topics, the research could be further strengthened by performing following experiments

- In this work, all the fluorescence spectroscopic analyses were carried out at constant 20^{o}C temperature. However, the work should be extended at different temperatures to provide additional chemical structural information of leachate DOM (Baker, 2005).

This could permit the use of thermal fluorescence properties for leachate quality monitoring in the treatment processes.

- Fourier Transform Infrared (FTIR) analyses of leachate samples needs to be done. This analysis could serve as a qualitative monitoring of chemical groups and bands of leachate DOM and provide more information about the structural changes of leachate DOM evolution (Kang et al., 2002; Huo et al., 2008).

- Synchronous fluorescence spectra (SFS) could also be carried out to explore additional structural information with improved resolution for leachate DOM which sometimes quite difficult to interpret clearly in the EEM spectra (Chen et al., 2003; Zhang et al., 2008).

- In the BMP test, compositional analyses of solid wastes were determined at the beginning and at the end of the experiment. Further research is needed to evaluate the compositional characteristics of wastes at the different stages of biodegradation processes. This would help to evaluate the relationship between the concentration of recalcitrant organic compounds in leachates and the degradation of waste components.

Appendix A

EEM Spectra of Landfill Leachates

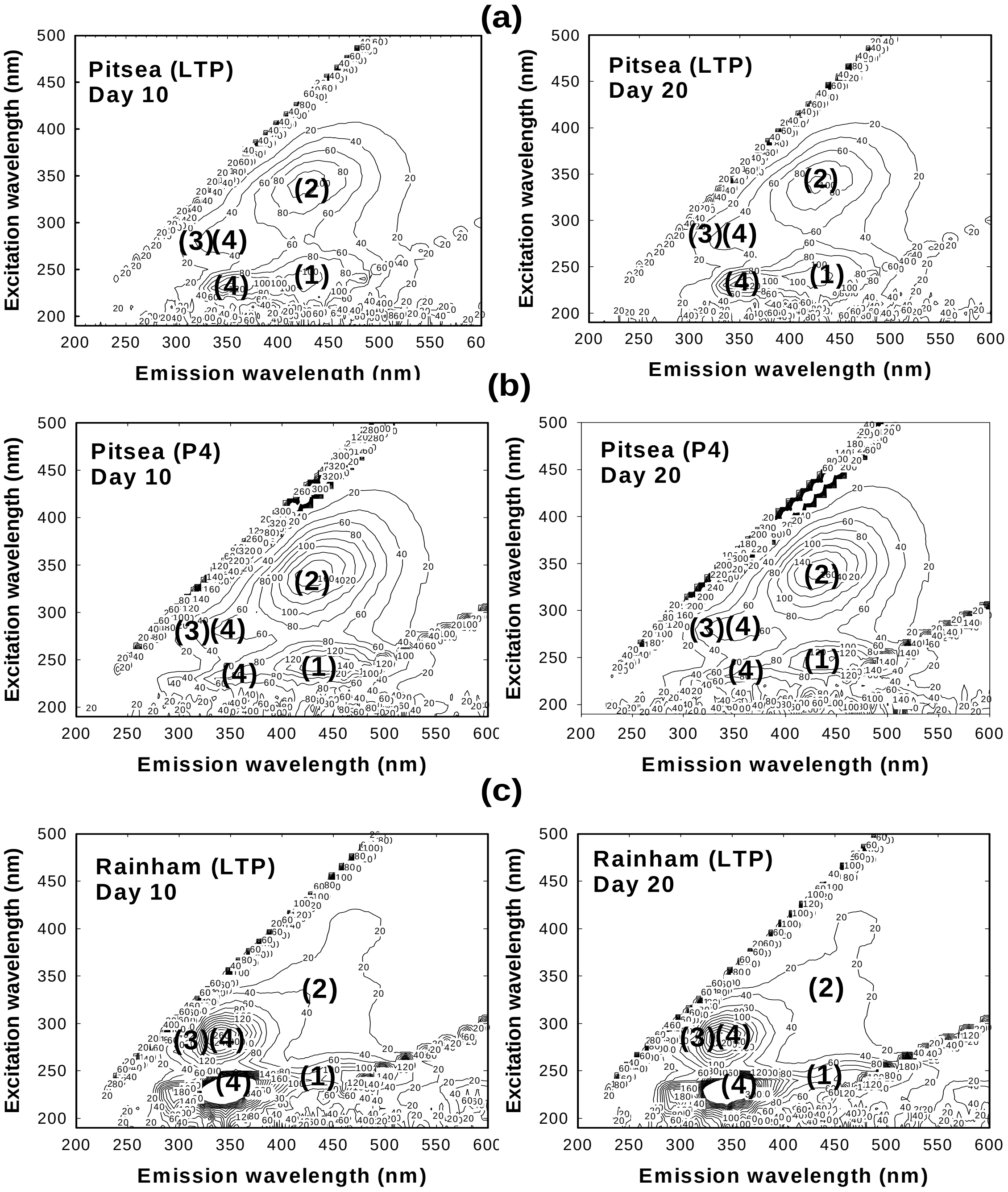


Figure A1 Excitation-emission matrices (EEM) for landfill leachates at day 10 and 20 during aeration (Zone 1 H-L; 2 F-L; 3 and 4 protein like) (dilution factor 25)

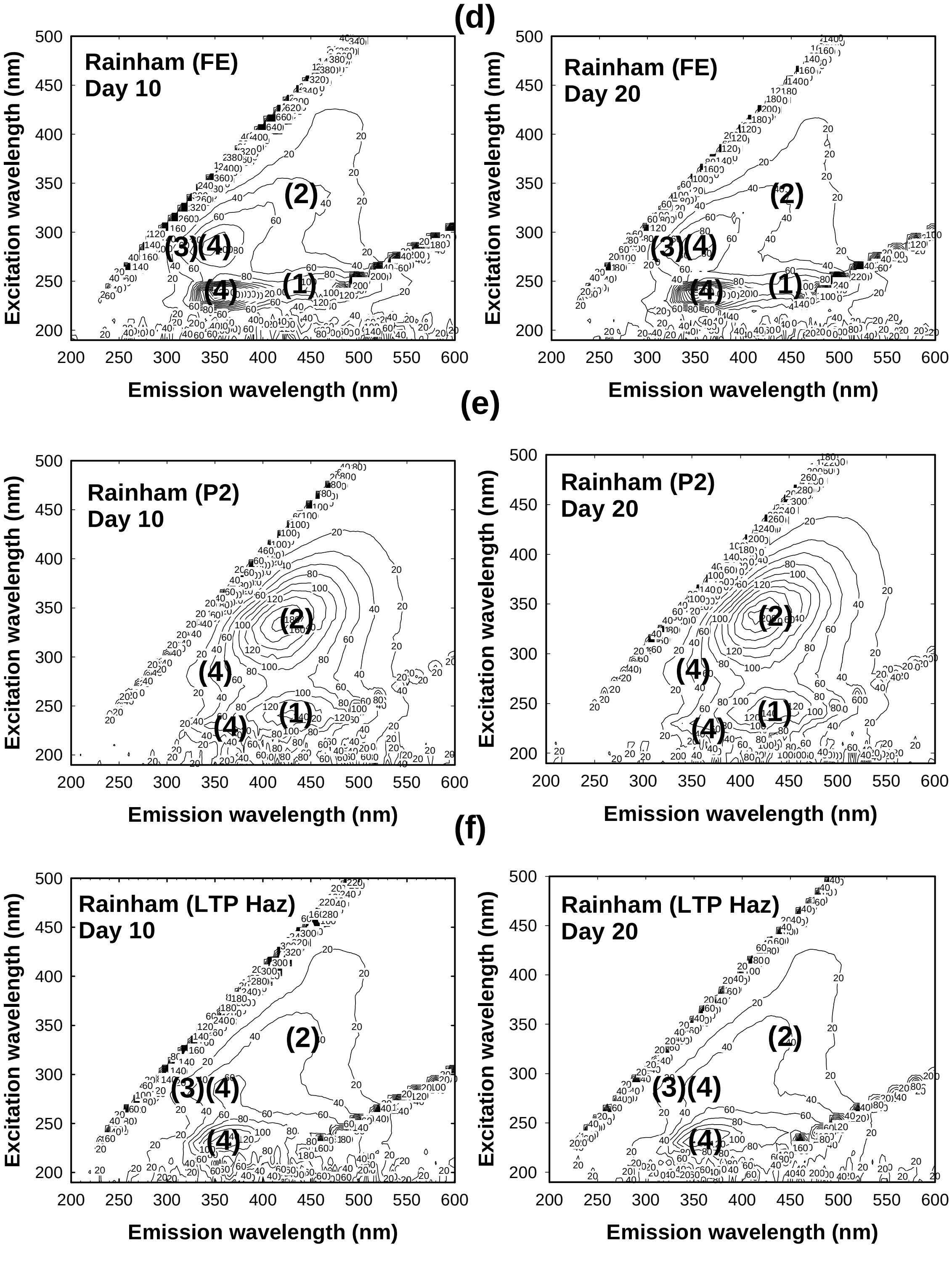


Figure A1 (Contd.) Excitation-emission matrices (EEM) for landfill leachates at day 10 and 20 during aeration (Zone 1 H-L; 2 F-L; 3 and 4 protein like) (dilution factor 25)

Appendix B

EEM Spectra of leachates from BMP reactors

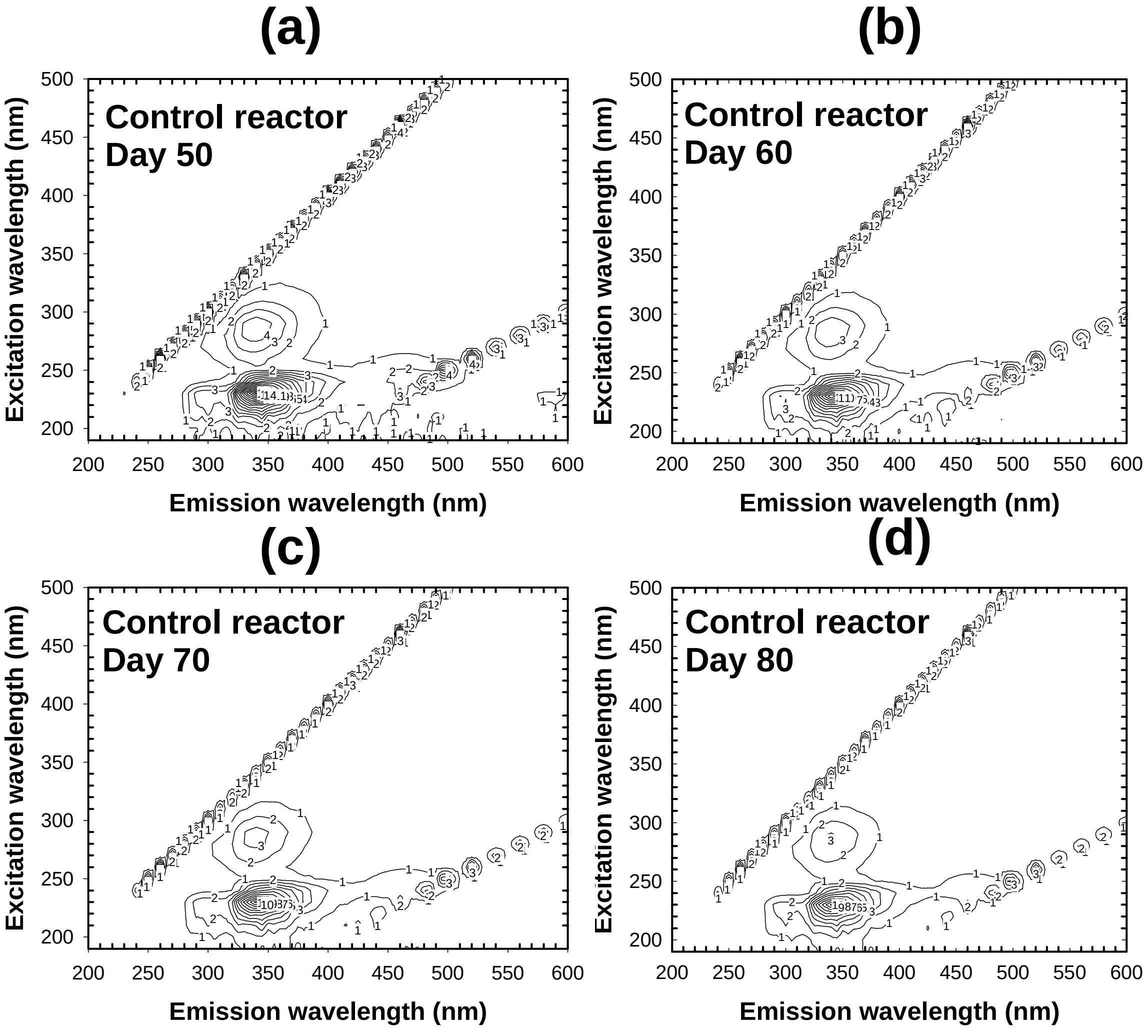


Figure B1 3D EEM fluorescence spectra of control (blank) reactors at day (a) 50, (b) 60 and (c) 70 and (d) 80 showing Trp-L fluorescence at 220-240 nm and 270-280 nm excitation and 340-370 nm emission (dilution factor 25)

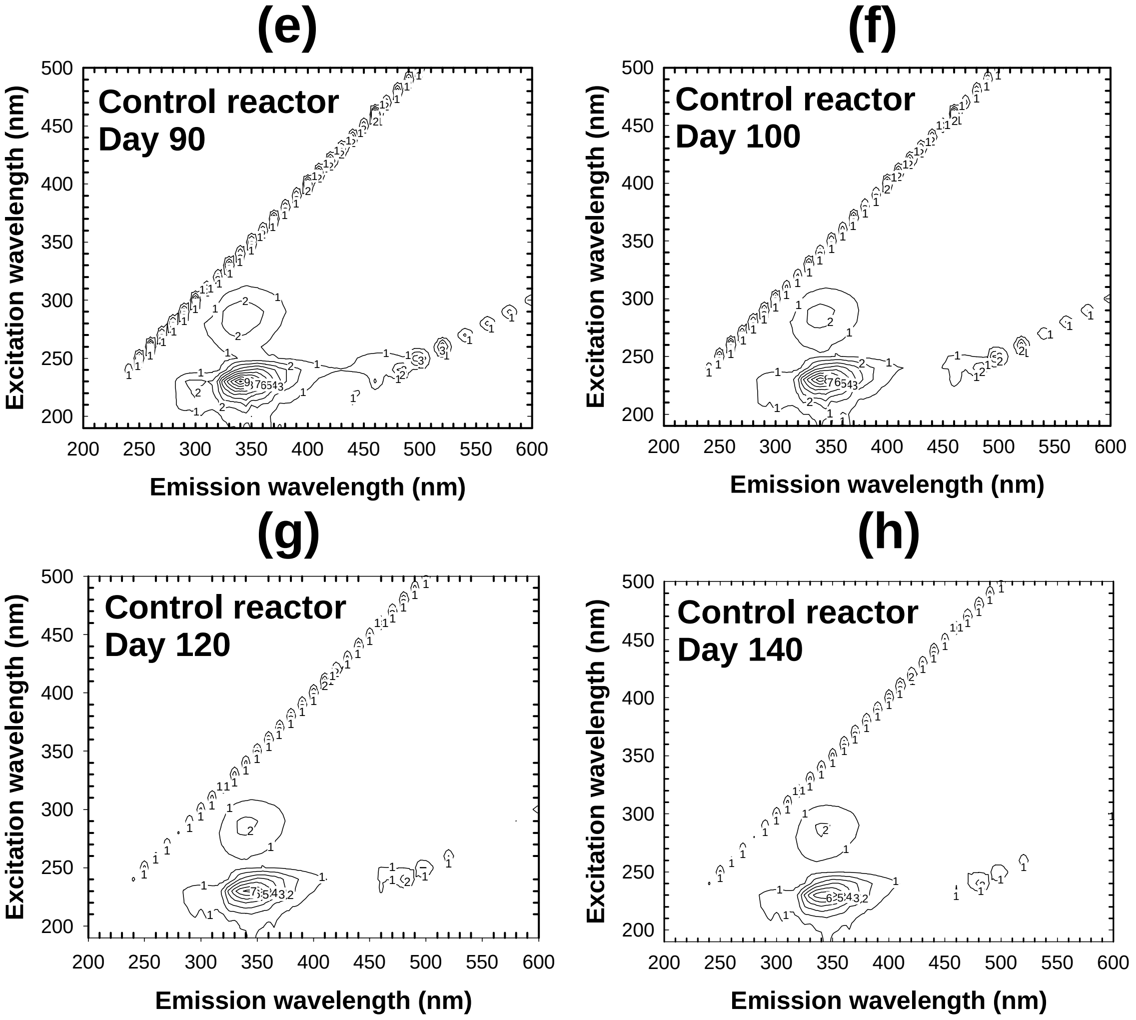


Figure B1 (contd.) 3D EEM fluorescence spectra of control (blank) reactors at day (e) 90, (f) 100 and (g) 120 and (h) 140 showing Trp-L fluorescence at 220-240 nm and 270-280 nm excitation and 340-370 nm emission (dilution factor 25)

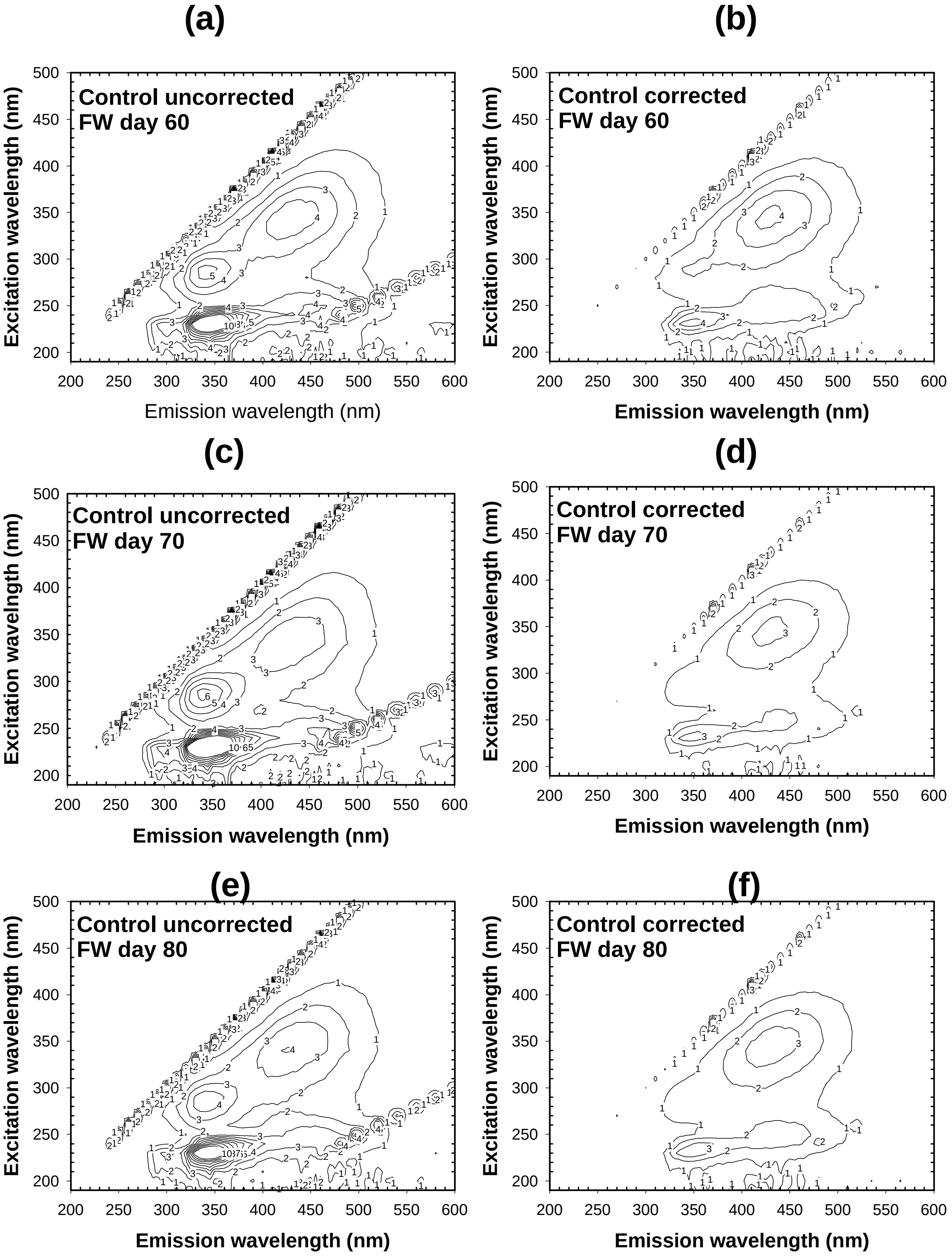


Figure B2 3D EEM fluorescence spectra of fresh waste (FW) leachates at day 60, 70 and 80 for control uncorrected and corrected samples (dilution factor 25)

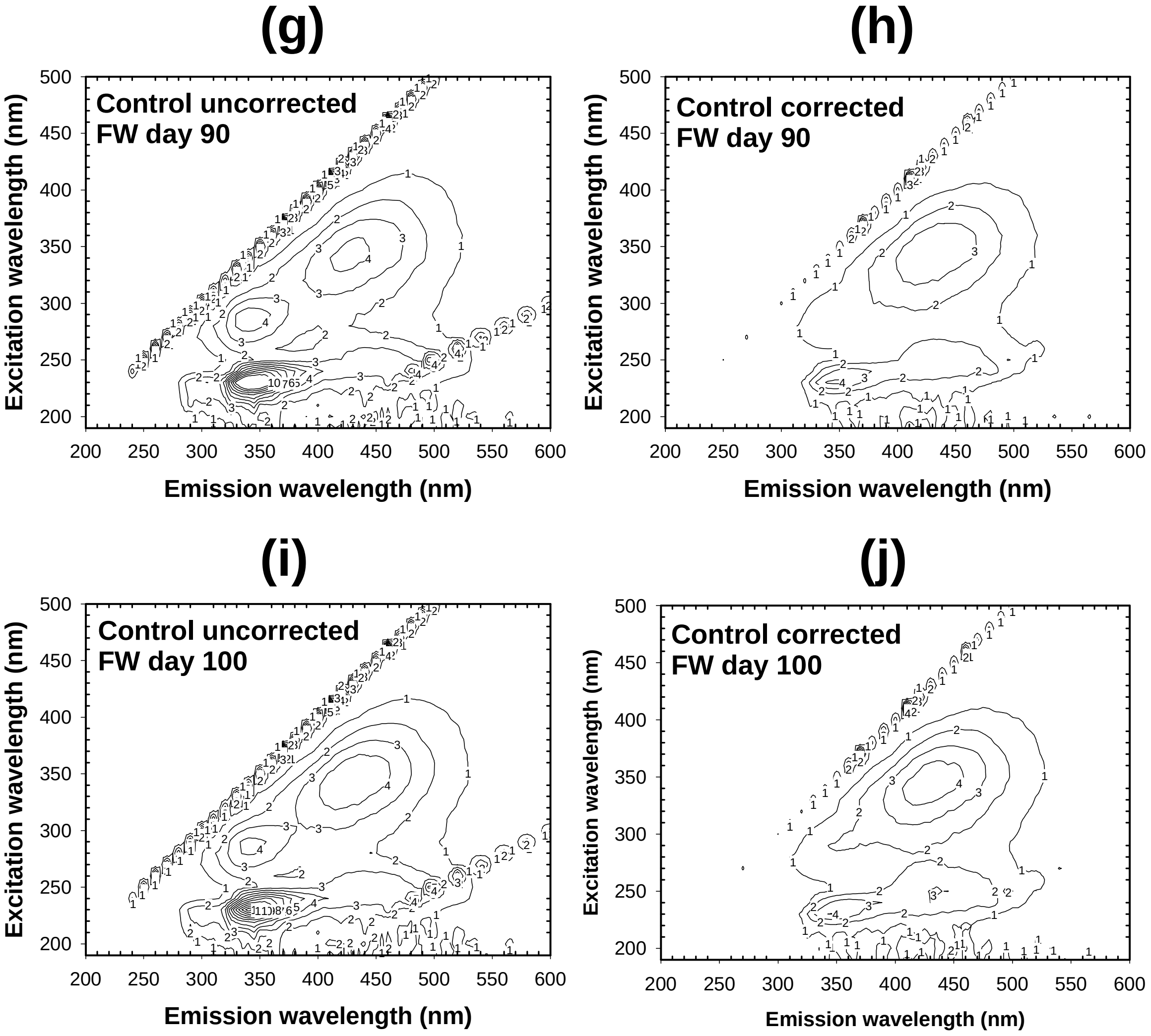


Figure B2 (contd.) 3D EEM fluorescence spectra of fresh waste (FW) leachates at day 90 and 100 for control uncorrected and corrected samples (dilution factor 25)

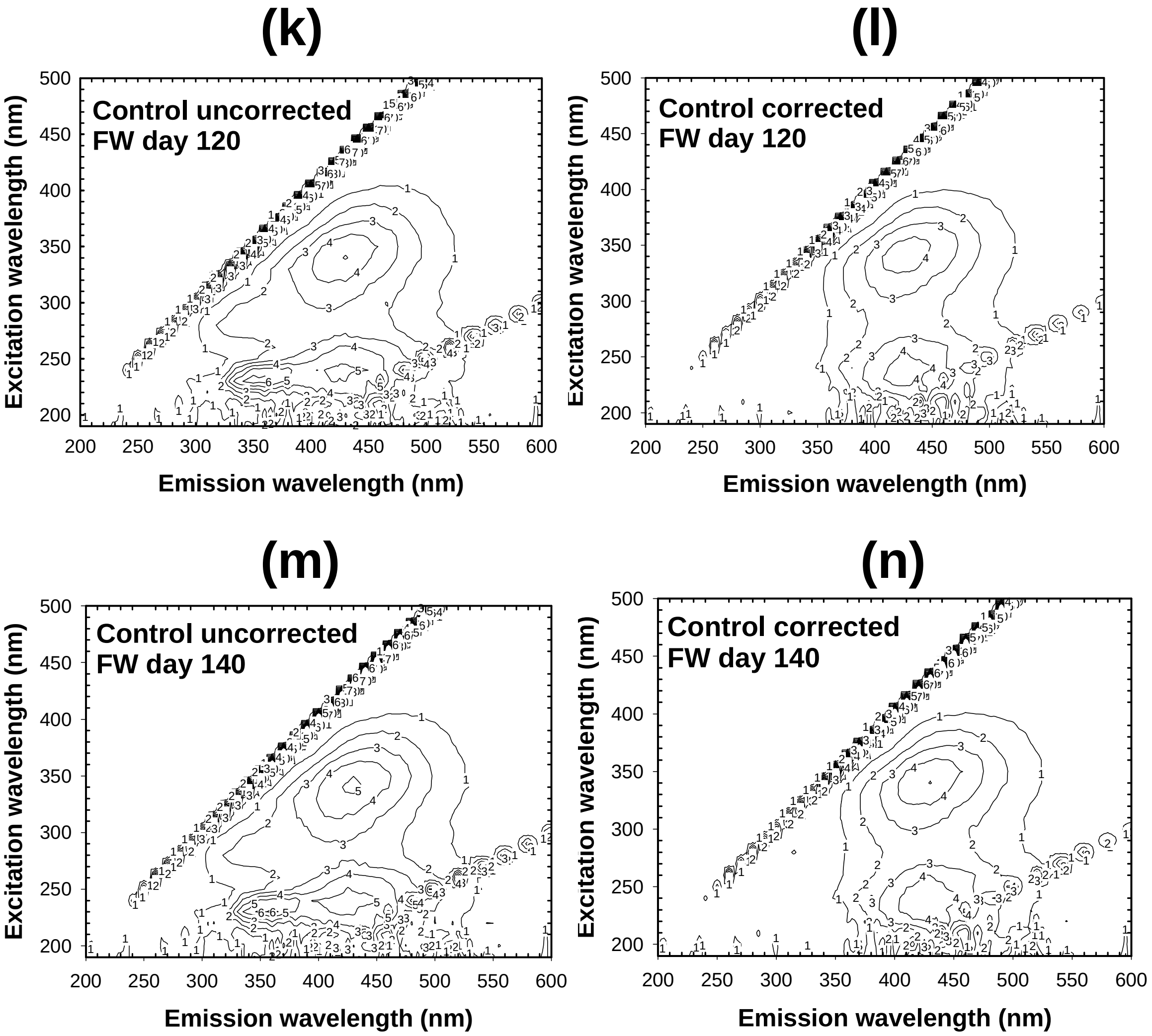


Figure B2 (contd.) 3D EEM fluorescence spectra of fresh waste (FW) leachates at day 120 and 140 for control uncorrected and corrected samples (dilution factor 25)

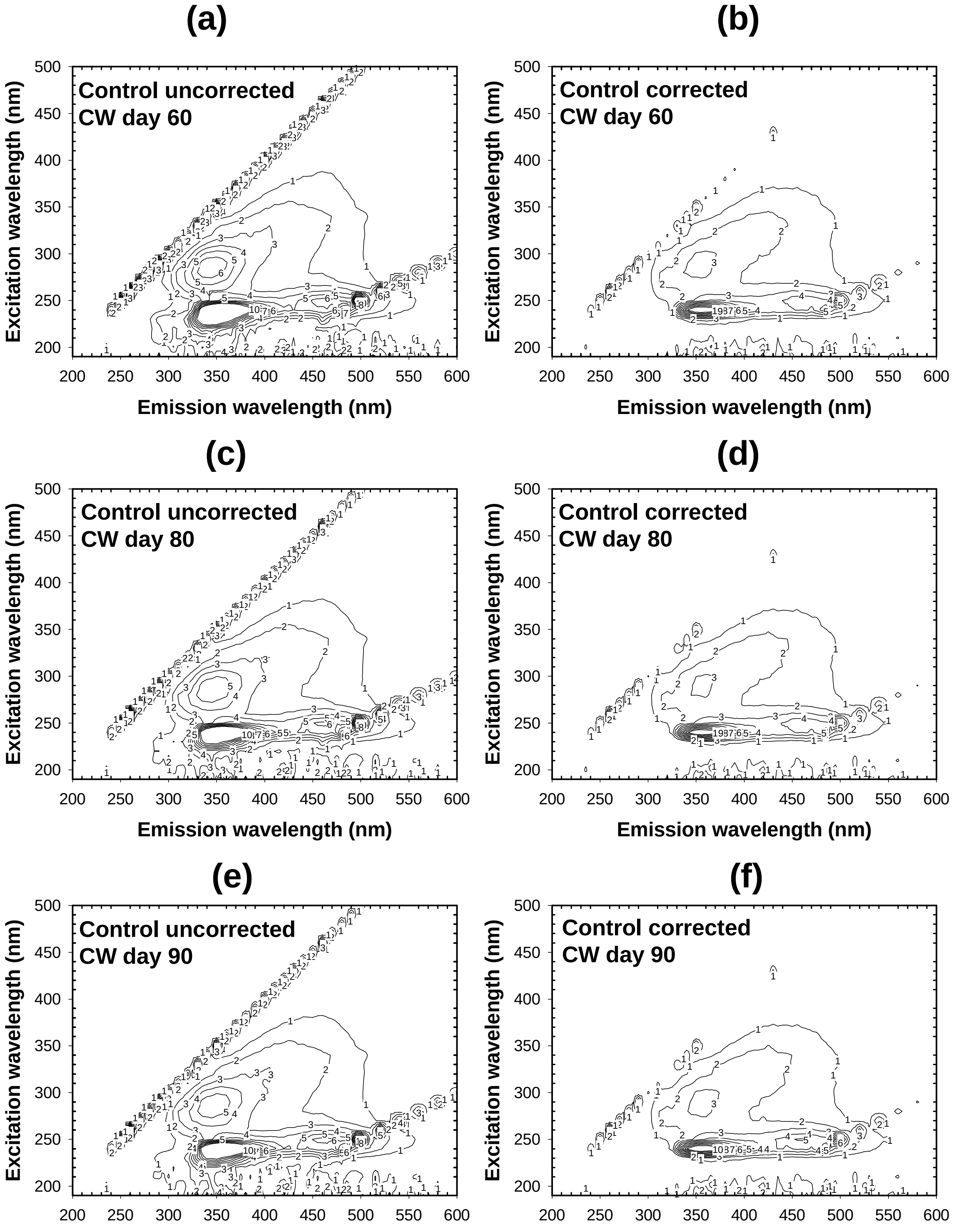


Figure B3 3D EEM fluorescence spectra of composted waste (CW) leachates at day 60, 80 and 90 for control uncorrected and corrected samples (dilution factor 50)

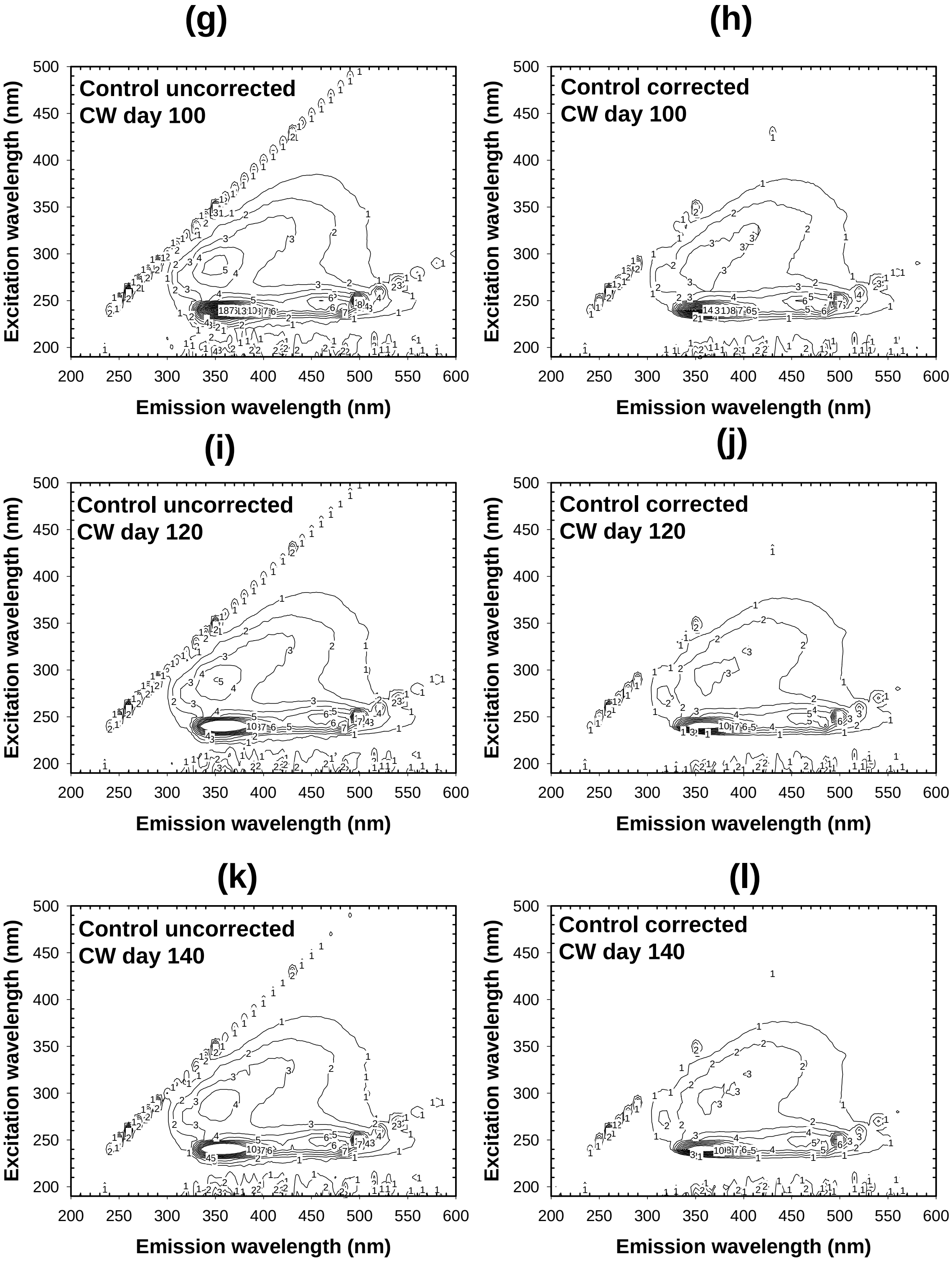


Figure B3 (contd.) 3D EEM fluorescence spectra of composted waste (CW) leachates at day 100, 120 and 140 for control uncorrected and corrected samples (dilution factor 50)

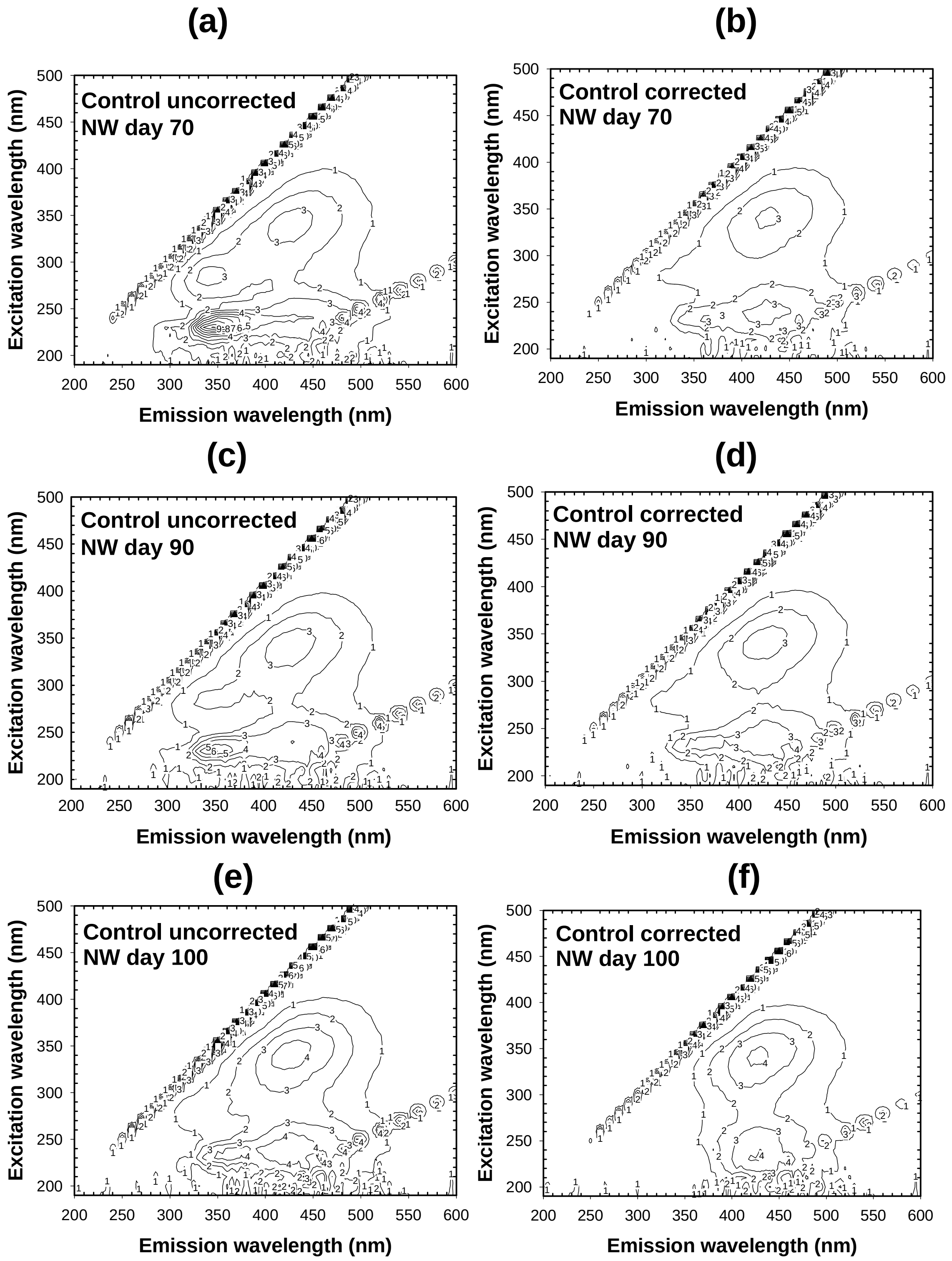


Figure B4 3D EEM fluorescence spectra of newspaper waste (NW) leachates at day 70, 90 and 100 for control uncorrected and corrected samples (dilution factor 40)

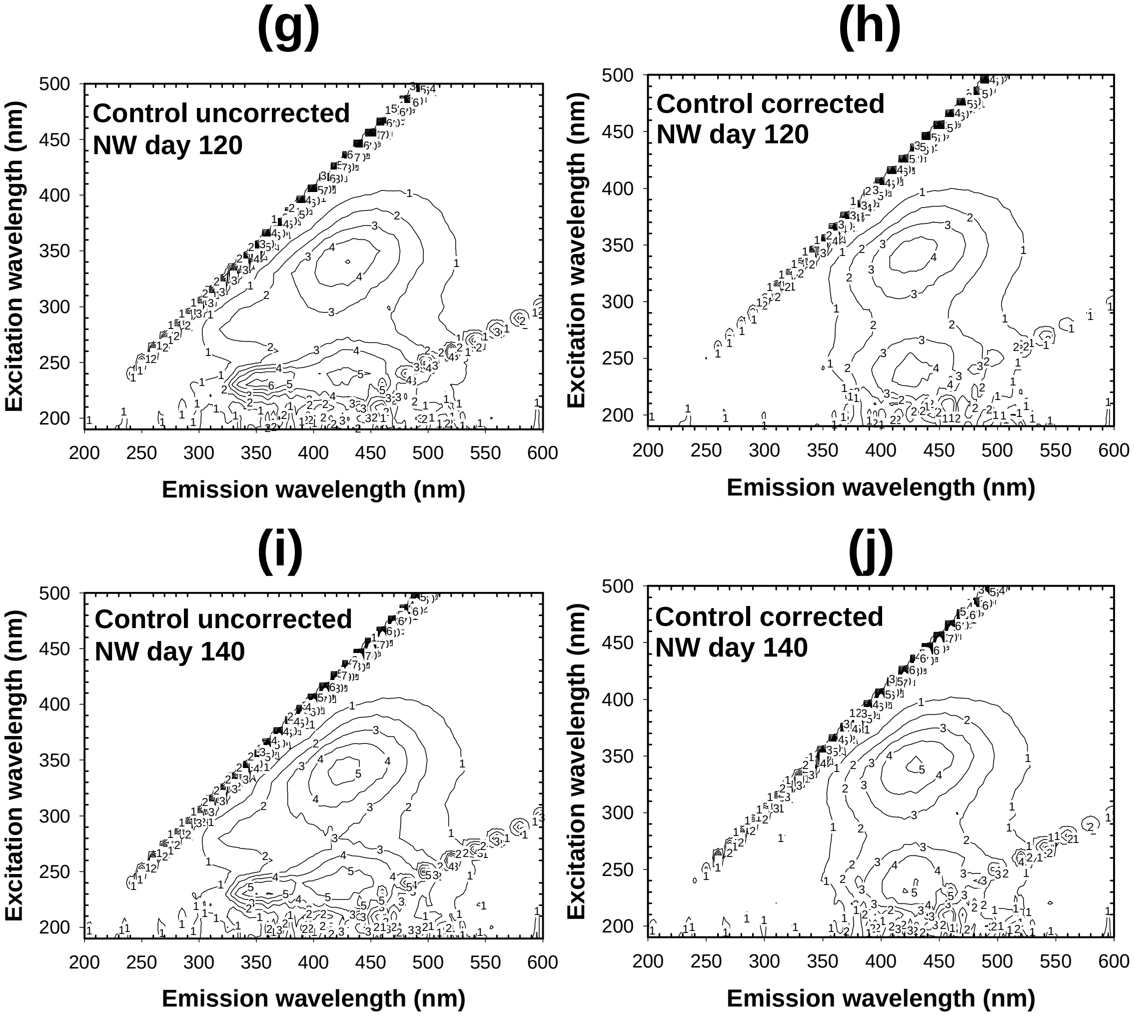


Figure B4 (contd.) 3D EEM fluorescence spectra of newspaper waste (NW) leachates at day 120 and 140 for control uncorrected and corrected samples (dilution factor 40)

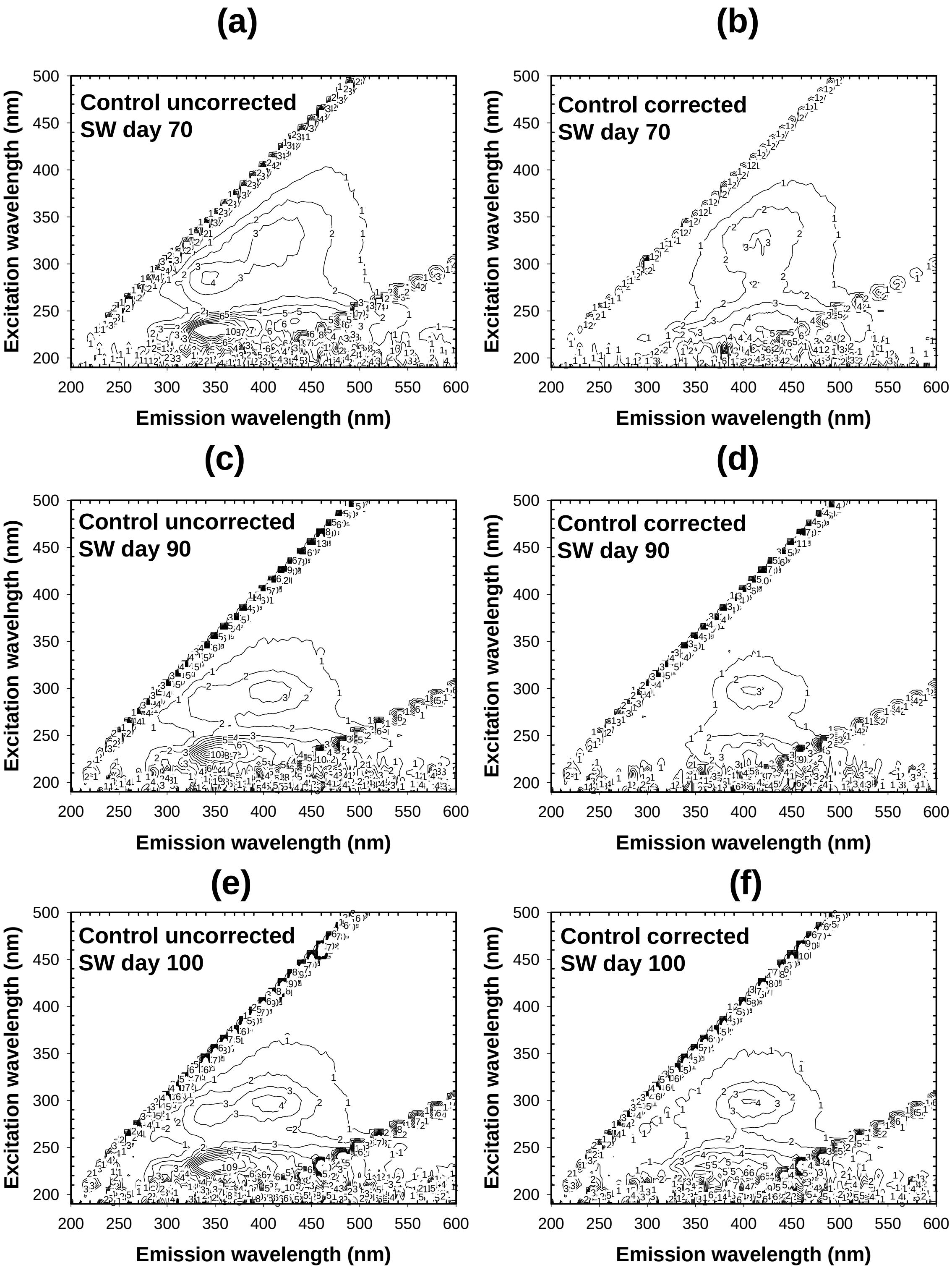


Figure B5 3D EEM fluorescence spectra of synthetic waste (SW) leachates at day 70, 90 and 100 for control uncorrected and corrected samples (dilution factor 10)

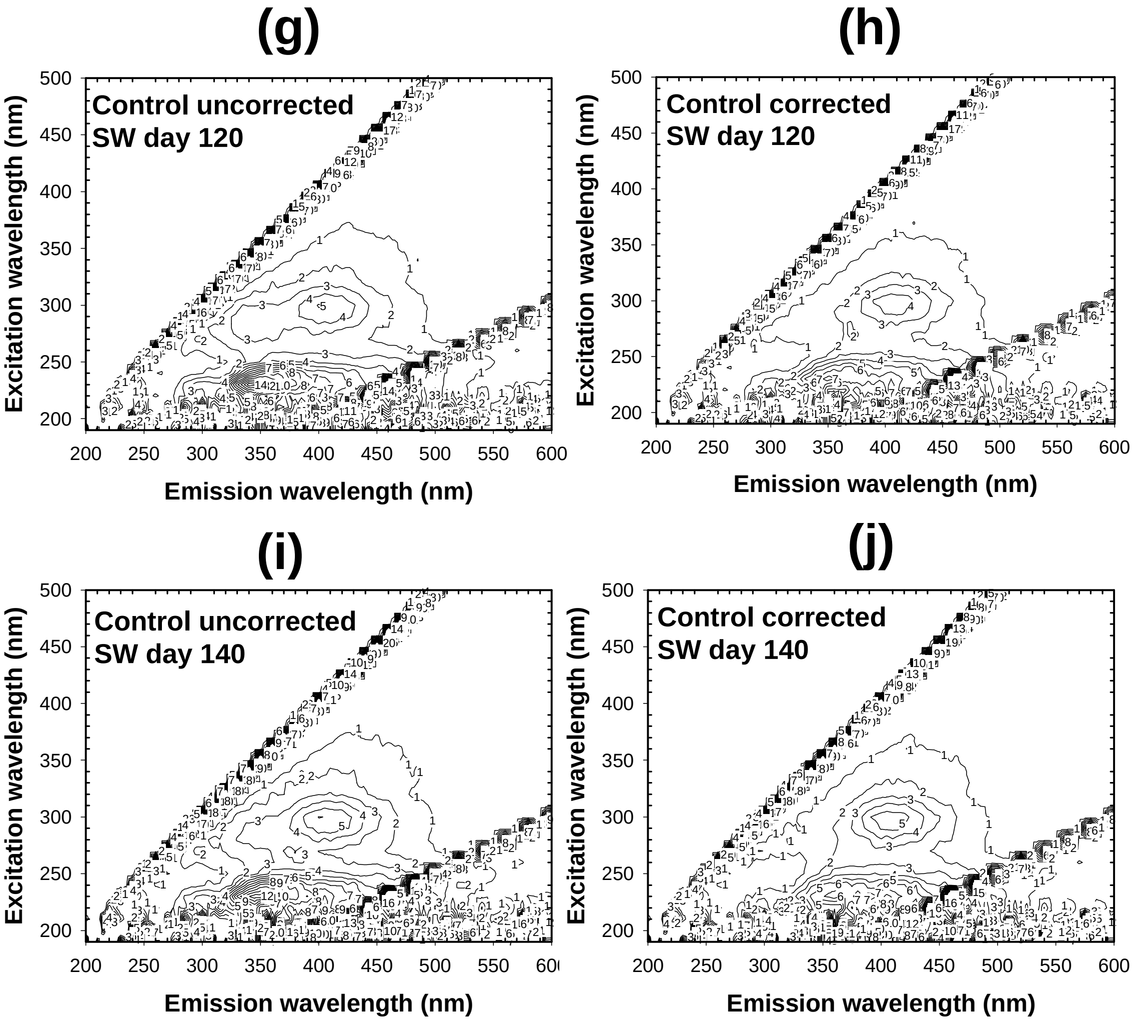


Figure B5 (contd.) 3D EEM fluorescence spectra of synthetic waste (SW) leachates at day 120 and 140 for control uncorrected and corrected samples (dilution factor 10)

References

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